GASTROINTESTINAL TRANSIT AND BIOAVAILABILITY OF A NOVEL SUSTAINED RELEASE THEOPHYLLINE FORMULATION

by

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ABSTRACT

A novel multiunit sustained release theophylline preparation has been formulated and assessed. Spherical pellets containing 80% theophylline were formed using an extrusion/spheronisation technique before being coated with an ethylcellulose-methylcellulose mixture using a fluidized bed coater. *In-vitro* dissolution studies established that satisfactory pH-independent release profiles were achieved, and that the rate of drug release could be varied in a predictable manner by manipulating the coat thickness. With additional thermal treatment of the coat, drug release was stable after storage of the products for one year. *In-vivo* evaluation of the preparation in healthy human volunteers, produced serum concentration profiles that were reflective of a controlled and sustained drug release, with complete bioavailability. A satisfactory correlation was also obtained between *in-vitro* and *in-vivo* results. Further comparison with a commercial preparation, Uniphyllin®, showed that the two products were bioequivalent when dosed fasted. In the fed mode, the rate of theophylline absorption from Uniphyllin was significantly increased, whilst that of the novel preparation was essentially unaffected, although a slight delay in absorption was noted. However, for neither preparation was the amount absorbed influenced by food status. Gastrointestinal transit studies of the novel preparation using a gamma-scintigraphic technique, revealed that the presence of food delayed the gastric emptying, but was without influence on the small intestinal transit time. The delay in gastric emptying was associated with a delay in drug absorption. For both fed and fasted states, the rate of absorption whilst the pellets were in the stomach was slower than when the pellets were in the small intestine. The pellets were less well dispersed in the stomach than in the small intestine or colon. Moreover, whereas only 14% of drug was released in the stomach, 46% was released in the small intestine. It is interesting that the remaining 40% of the drug was taken up from the colon, which thus acts as a significant site of absorption.

To my lovely wife Dr Ooi Kheng YONG

and my cute little son Chee Wah YUEN

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1.1 ORAL SUSTAINED RELEASE DOSAGE FORMS

1.1.1 GENERAL PRINCIPLES

In view of its convenience to the patient, oral administration is still the route of choice for the administration of most drugs. Ideally, an oral dosage form should deliver the drug to its site of action, at the minimum rate required to elicit the desired therapeutic response over the duration of the dosing interval. Since blood is usually the medium of transport for the absorbed drug, this ideal is best accomplished by providing a plasma-concentration profile which produces optimal therapeutic activity. Unfortunately, this goal can only be partially achieved with conventional dosage forms (*Lee and Good*, 1987).

To maximize drug availability, conventional dosage forms are generally designed so that the rate and extent of absorption are maximal. Wide fluctuations in peak and trough steady-state drug levels are often obtained with these products in multiple dose administration, particularly if the biological half-life of the drug is short. Such fluctuations are undesirable with drugs of narrow therapeutic indices. Whilst increasing the frequency of dosing may be able to reduce these fluctuations, it may also lead to patient inconvenience and poor compliance. Because of these shortcomings, a number of approaches have been used to formulate sustained release dosage forms. To be effective such formulations must control the rate of oral drug release for an extended period of time after each administration.

One of the earliest attempts to control drug release in the gastrointestinal tract was the use of enteric coating. In 1930, Wruble formulated an acid resistant coating material comprising a mixture of lac gum, alcohol and ammonia, and the effectiveness of the coating was borne out by both *in-vitro* and *in-vivo* experiments. Other enteric coating methods were also described at around this period by Husa and Magid (1932), Mills (1937), and Goorley and Lee (1938). However, these early enteric coated products were formulated primarily to delay drug release until emptying from the stomach had occurred. Among the earliest modern sustained release products was the 'Spansule' capsular dosage form marketed by Smith, Kline and French Laboratories in the 1950's (US Patent No. 2738303). The introduction of the 'Spansule' concept was an overwhelming success, and truly represented one of the milestones

of pharmaceutical technology (*Shangraw* 1988). It also marked the beginning of the era of sustained drug delivery during that period. Numerous sustained release products began to appear on the market, and their mode of actions were described by various names such as 'sustained action', 'prolonged action', 'long acting', 'timed released', and 'extended action' (*Ballard and Nelson*, 1970).

The design and formulation of the early sustained release products in the 1950s were not without problems. At this time, the science of biopharmaceutics and pharmacokinetics was still in its infancy. As a result, these early products were formulated with little or no consideration of the absorption and disposition characteristics of the drug in the body. Also, the lack of appropriately sensitive analytical techniques at that time, presumably made evaluation of the blood levels impossible. Thus the efficacy of these formulations could only be assessed by pharmacological methods which were often unreliable or even inapplicable to the drug under test. As the field of biopharmaceutics and pharmacokinetics was better developed, the shortcomings of these old products became evident. Some of the old products were eventually withdrawn and improvements were made in the newer formulations. Towards the end of 1960's, a new term, controlled drug delivery, came into being. This term was applied to those formulations in which the rate of dissolution of drug from the dosage forms was controlled. Such products have enchanced bioavailability, efficacy and safety (*Banker* 1979).

Significant progress has been made in this area in the past two decades, and today, sustained release formulations have become an important product line of most major drug companies. A variety of techniques have been used in the formulation of these products but they all basically work on the same principle of slowing the rate of dissolution or release of the drug from the dosage form. In general, there are two sets of methods to achieve this objective (*Lordi*, 1986). The first set is based on modification of those physical and/or chemical properties of the drug that affect bioavailability. These include the use of complex formation (example tannate complexes), ion-exchange resins to form drug adsorbates, and prodrug synthesis. The mechanism of sustained drug release is through decreased rate of dissolution of the altered drug and/or dissociation of the free drug into solution. In the case of prodrugs, a slow regeneration of the parent compound after absorption, may provide an additional strategy for prolonging the drug action. A major advantage of this approach is that it operates

independently from the dosage form. The resulting drug modifications can thus be formulated as liquid suspensions, capsule or tablet. However, these methods can only be applied to drug moieties containing the appropriate functional groups.

In contrast, the second set of methods are based on modification of the drug release characteristics of the dosage form. Although products based on dosage form modification are of many designs and constructions, the mechanisms underlying the sustained release are few (*Lee and Robinson*, 1978). A drug with a slow dissolution rate is inherently sustained. For those drugs with rapid dissolution, embedding them within a slowly dissolving or erodable matrix provides a means of retarding the dissolution rate. Various lipid materials, waxes and other polymers have been found to be useful for this purpose. Drug release occurs through a combination of leaching of the active substance from the matrix and erosion of the matrix material. Another approach is to disperse the drug within an insoluble matrix. The most common insoluble matrix is one which is porous in nature, containing small channels and passages which are filled with drug. *In-vivo*, fluids from the gastrointestinal tract penetrate and dissolve the drug, and the dissolved molecules then diffuse from these passages out of the matrix. Drug release is thus delayed as the dissolved molecules have to diffuse through a network of capillaries between the compact polymer particles forming the matrix. The kinetics of release from such matrices have been well studied by Higuchi (1963).

Coating the drug particles or pellets with a barrier membrane is another effective means of controlling the drug release. The barrier coat can either be slowly soluble or insoluble in nature. In the former case, the pellets release their contents through erosion of the coat. A typical product utilizing this release mechanism may consist of a capsule containing numerous pellets coated to various thicknesses with some erodable material. Since the rate of erosion of the coat can be expected to be dependent on the coat thickness, such a product will yield a relatively continuous drug release. The 'Spansule' dosage form discussed earlier was based on this design (*Blythe*, 1956). A variation of this method is to coat the pellets with different coating materials of different dissolution or disintegration times, or successively coating a spherical pellet, in between which, is placed the active drug (*Hermelin*, 1957). A second mechanism whereby coated pellets release their medicaments is by diffusion of the drug through the intact coat. Following ingestion, moisture within the gastrointestinal tract penetrates the coat to dissolve the solid drug. The dissolved drug molecules then diffuse

through the intact barrier membrane. The rate of drug release can be controlled by varying the nature and/or thickness of the coat or by altering its porosity by incorporating some water soluble materials into the coat to act as channeling agents. It is interesting to note that osmosis has recently been suggested as an important mechanism for the drug release from such systems (*Zentner et al*, 1985; *Ozturk et al*, 1990; *Lindstedt et al*, 1989, 1991).

Formulations in which osmotic pumping is a major release mechanism were described by Theeuwes in 1975. In this approach, the osmotically active solid, namely the drug or dispersion thereof is surrounded by a rigid rate-controlling membrane which is semipermeable with respect to water. Uptake of water through the rigid membrane at a controlled rate, will cause the device to deliver, via an orifice in the membrane, a volume of saturated drug solution equal to the volume of water imbibed. The rate of drug release is constant so long as excess solid remains within the device. Because the mechanism of this system is based on osmotic pressure, the system delivers drug at a rate that is essentially independent of stirring rate and the environmental pH. A commercial therapeutic system based on the above principle has been marketed by Alza Corporation USA, under the name 'OROS', and its application has recently been examined with oxprenolol (*Bradbrook et al*, 1985), metoprolol (*Godbillon et al*, 1985a) and nifedipine (*Chung et al*, 1987). Some clinical problems were encountered in its use with indomethacin, resulting in the withdrawal of the product, and this has been well reviewed by Bem *et al* (1988). Recently, Davis *et al* (1988) have also investigated the gastrointestinal transit properties of the 'Oros' containing the drug oxprenolol. The rate of appearance of the drug in the systemic circulation in relation to the location of the dosage in the gut was examined.

1.1.2 ADVANTAGES

Several therapeutic advantages can be gained by using sustained release formulations. One such advantage is in prolonging the duration of action of the drug beyond that normally achieved with conventional dosage forms. Consequently, the frequency of dosing can be reduced to once or twice daily leading to improved patient convenience and compliance. Indeed, good compliance has been recognized as an important element in successful drug therapy. Several studies have shown that poor patient compliance is observed in self-administration when the number of drugs and daily doses are increased (*Blackwell*, 1973; *Haynes*, 1981; *Mucklow*, 1979; *Parkin et al*, 1976; *Porter*, 1969), and appears commonest

when three or more daily doses are prescribed (*Ayd*, 1974; *Gatley*, 1968).

Another important benefit is the attainment of a relatively constant steady state blood level with reduced peak-trough fluctuations, thereby achieving better efficacy/toxicity ratio with the drug (*Theeuwes*, 1983; *Urquhart*, 1982). This is especially important with drugs of narrow therapeutic indices, since a smaller fluctuation will eliminate periods of supra-therapeutic levels associated with systemic toxicity, and avoid sub-therapeutic levels resulting in loss of therapeutic efficacy. Clearly then, there is better disease management with sustained release dosage forms and the overall reliability of therapy is accordingly increased (*Urquhart*, 1981). Viewed from a broader perspective, this would mean a total cost savings in health care, in terms of fewer lost work days, less hospitalization and decreased number of visits to the physician (*Lee and Robinson*, 1978).

Elimination of local irritation and erosion arising from exposure of the gastric mucosa to high drug concentrations have also been reported for individual drugs, examples are aspirin (*Levy and Hayes*, 1960), and aminophylline (*Boroda et al*, 1973). However, this point remains controversial. Perforations of the small bowel was reported with the 'OROS' controlled release formulation of indomethacin, but it is still unclear whether this was due to the local effect of indomethacin, the osmotic agent or a systemic effect related to constant indomethacin plasma levels (*Brors*, 1987; *Shaw*, 1988)

1.1.3 MULTIUNIT VERSUS SINGLE UNIT

Oral sustained release preparations may be classified as single unit or multi-unit dosage forms (*Bechgaard and Nielsen*, 1978). The single unit system may consist of a single tablet such as a matrix tablet while the latter may comprise many small pellets contained in a hard gelatin capsule. On the basis of this difference, the multi-unit dosage form appears to have several advantages over the single unit system. The pellets are usually spherical in shape and attractive in appearance. Individual units are easily coated with a rate controlling membrane which can be varied in nature and thickness to give more effective release patterns. Moreover, a multi-unit system permits easy dose adjustment by varying the number of pellets in each capsule. Since each dose consists of many subunits, there is better statistical assurance of drug release (*Lordi*, 1986), and the risk of dose dumping is equally subdivided (*Beckett*, 1985). Thus, ineffective plasma level of drug due to incomplete release of the

medicaments is less likely to occur with a multi-unit system (*Murthy et al*, 1983).

Since individual pellets have the potential to distribute evenly and widely over a large area in the stomach and small intestine, it was asserted that this dispersion process would yield a more predictable drug release profile by reducing the local differences in the gastrointestinal environment (*Murthy et al*, 1983; *Beckett*, 1984). Furthermore, local effects of an irritant drug could similarly be reduced (*Rowe*, 1983). This dispersion property was however, disputed by Hunter *et al* (1982), Wilson *et al* (1983) and Devereux (1987). Another potential advantage, is the difference in the gastric emptying pattern of the two types of dosage forms. Bechgaard (1982) suggested that since gastric emptying of a single unit dosage form was essentially a random process, there would be an inherently large intra and inter-subject variation. In contrast, a multi-unit system was emptied more gradually with small individual variations giving better predictability for a given dose. It was presumed that the subunits were small enough to pass through the contracted pylorus. Previously, it was suggested that only subunits of less than about 2mm, would empty gradually, but recent investigations have indicated that larger units may also empty gradually from the stomach (*Feely et al* 1987; *Feely and Davis* 1989). The critical size was suggested to be 3mm (*Meyer* 1989). Although Beckett (1983) proposed a linear gastric emptying pattern for multi-unit dosage forms, Hunter *et al* (1982) and Devereux (1987) suggested that this pattern may not always be obtained and that the pellets may at times empty as a single bolus.

1.1.4 PHYSIOLOGICAL INFLUENCE ON PERFORMANCE

The human gastrointestinal tract is a very complex organ, which can be divided into three distinct sections, namely the stomach, the small intestine and the colon. Each has its own physiological function and is very varied in terms of pH, nature of its luminal contents, length and surface area. All these variables may singly or in combination influence the drug release from a sustained release preparation.

The small intestine, with its enormous absorptive area of between 200 - 500 m^2 (*Davenport*, 1977), is invariably the principal site of drug absorption. In contrast, the stomach, being a secretory rather than an absorptive organ, and the colon, because of its small absorptive area, usually play a small role in drug absorption. Nevertheless, particularly in the case of sustained release preparations, the colon may play an important part in drug absorption.

Although some drugs, for example theophylline (*Staib et al*, 1986) and metoprolol (*Godbillon et al*, 1985b) have been shown to be well absorbed in the colon: in general, absorption from this part of the intestine is incomplete and erratic (*Koch-Weser and Schechter*, 1981), since transit times through the colon are highly variable (*Metcalf et al*, 1987) ranging from less than an hour to more than sixty hours (*Hardy et al*, 1985, 1987). Absorption from the distal part can be considered negligible since any remaining drug will be embedded in semi-solid faecal matter (*Hirtz*, 1984).

In view of the differences in the local environment and absorptive capacity of the three sections of the gastrointestinal tract, the duration of residence and transit times of a sustained release product in each section, can greatly affect its performance. Even if a product can be formulated to release its medicament independent of the local environment, its performance can still be influenced by the orocaecal transit time if the drug is not absorbed in the colon.

The transit time through the small intestine typically takes three to five hours, is fairly constant, and is unaffected by food status (*Cammack et al*, 1982; *Davis et al*, 1984, 1986a, 1987; *Ollerenshaw et al*, 1987; *Devereux* 1987; *Mundy et al* 1989). On the other hand gastric emptying is very variable, and is influenced by diet (*Christian et al* 1980; *Davis et al*, 1984, 1987; *Moore et al*, 1981; *Devereux*, 1987), drugs (*Kaus et al*, 1984; *Lake-Bakaar and Teblick*, 1984), posture (*Bennet et al* 1984; *Fell et al*, 1982; *Hunter et al, 1982*) and also exercise (*Cammack et al*, 1982). It follows that, the orocaecal transit time is highly dependent on gastric emptying. Hence, by prolonging the gastric residence, the overall transit time of a dosage form can therefore be extended. If the drug dissolves in the stomach contents, drug solution will then pass in an unimpeded manner to the small intestine for subsequent absorption at the optimal site.

To date, several techniques have been used to prolong gastric emptying time. Although Ch'ng *et al* (1985) achieved promising results with bioadhesive polymers in rats, they were found to be ineffective in humans (*Khosla and Davis*, 1987). Similarly, when Groning and Heun (1984) incorporated myristic acid, a known gastric emptying inhibitor into their tablet formulation, this was also unsuccessful. The effect of particle size was examined by Gruber *et al* (1987), Hinder and Kelly (1977), Holt *et al* (1982), Meyer (1980) and Meyer *et al* (1981, 1985). Although delayed emptying was achieved with particles of larger sizes (*Kaniwa*

et al, 1988a, 1988b), the retention time was dependent on the food status (*Davis et al*, 1986b).

Another interesting approach was that of modifying the density of the dosage form. Using a variety of different substances, Hoezel (1930) provided the first evidence that the total transit time could be extended by increasing the density of the ingested material. However, results from subsequent studies which used high density dosage forms have produced differing outcomes. Their delayed emptying was reported by Kirwan and Smith (1974), Bechgaard and Ladefoged (1978), Meyer *et al* (1985, 1988), Sirois *et al* (1987), Takahashi *et al* (1985) and Devereux (1987). On the other hand, the emptying rate has also been found to be independent of density by Cummings *et al* (1976), Bogentoft *et al* (1981, 1982), Kaus *et al* (1984a), Bechgaard *et al* (1985), Davis *et al* (1986c), Kaniwa *et al* (1988b) and Gruber *et al* (1987). Most of the dosage forms studied however, were with densities of not more than 2.0 Gcm^{-3}, except for those examined by Devereux (1987) and Gruber *et al* (1987). In a more recent study, Clarke (1989) suggested that the critical density for delaying the gastric emptying may lie between 2.4 and 2.6 Gcm^{-3}. Formulations which floated on the gastric contents were investigated by Muller-Lissner and Blum (1981), Sheth and Tossounian (1984), Hou *et al* (1985), Stockwell *et al* (1986), Sangekar *et al* (1987) and Miyazaki *et al* (1988), but a major drawback of such devices is that the stomach must remain in a fed condition.

1.1.5 *IN-VITRO* AND *IN-VIVO* EVALUATION

The advent of sustained release formulations in the mid-1950's together with the growing realization that disintegration tests were no real index of bioavailability have provided the impetus for developing model *in-vitro* systems to study the actual drug release patterns of solid dosage forms. Subsequently, a wide variety of dissolution test systems were developed and described in the literature. Although it is not possible to simulate the whole range of variables that affect drug release in the gastrointestinal tract, a properly designed *in-vitro* test is not only invaluable in dosage form development, but also indispensable for quality control of the final proven formulation.

Smolen and Ball (1984) summarised some of the attributes that were essential for a good dissolution test system. Firstly, the results obtained must be reproducible and be correlated or associated with some characteristics of the *in-vivo* data. Secondly, the test should be sufficiently sensitive to discriminate between those *in-vitro* variables that yield *in-vivo*

differences, without being overly discriminatory in detecting *in-vitro* changes that are negligible *in-vivo*. Thirdly, the method should be flexible, and capable of application to a wide range of products, and finally, the procedure should preferably be amenable to automation.

In general, dissolution test systems have evolved into two distinct types, the stirred vessel and the flow through column systems (*Nelson and Miller*, 1979). The former is characterized by a relatively large dissolution volume with minimal liquid exchange, and agitation is accomplished by stirring the liquid using a rotating blade or by motion of the vessel itself. The second type usually consists of a relatively small dissolution cell through which fresh solvent flows at a constant rate with no additional agitation. Regardless of the type however, certain process variables are deemed essential to obtain meaningful results, and should be properly selected in the dissolution test methodology (*Smollen and Ball*, 1984). A suitable agitation intensity is an important requirement to enable discrimination of formulation parameters that are important *in-vivo*. The composition of the dissolution medium should be aqueous in nature and be buffered at different pH values in order to determine the pH dependency of drug release from the dosage form. This is necessary to mimic the variable pH of the gastrointestinal tract. Some formulations may contain retardants whose function depends on the presence of normal constituents of the gut (example bile salts, pancreatin or pepsin). For these products, the dissolution medium should be further refined to contain the appropriate materials. It is also important to maintain sink condition by keeping the concentration of the dissolved drug in the bulk medium below 15 percent of saturation (*Swarbrick*, 1970) and to conduct the test at 37 $\pm$ 0.5°C, as both of these factors can affect the rate of drug release.

The USP dissolution test uses either a rotating basket assembly (apparatus 1) or a rotating paddle system (apparatus 2). A survey of the literature reveals that one or the other of these USP dissolution test apparatuses have frequently been used to evaluate sustained release products. Both types are easy to operate and the procedures are readily automated. Other systems that have been used include the rotating bottle apparatus, Sartorius absorption and solubility simulator, and the column-type flow-through assembly (*Lordi*, 1986). With the exception of the rotating bottle apparatus, which was originally developed for evaluating sustained release products (*Krueger and Vliet*, 1962), the other two systems are also amenable

to automation and easy manipulation of dissolution media.

Whilst *in-vitro* techniques offer cheap and easy means for evaluation during dosage form development, validation of the final product can only be accomplished by *in-vivo* testing, and ultimately the most reliable test is to perform human studies. This usually entails a comparison of the blood level profile achieved with the dosage form, with that of a reference product of the same drug such as a solution administered orally or intravenously, or a proven conventional fast release formulation. The rate and extent of absorption of the dosage form can then be determined by relevant pharmacokinetic analysis of the data. In addition, it is necessary to establish bioequivalency of the dosage form with a proven, currently marketed product. Since food is known to influence the absorption of drugs, the effect of food status should also be assessed (*Karim*, 1988). All these studies are necessary to ensure that the dosage form meets its sustained release claims, that no dose dumping occurs, and sufficient bioavailability is achieved. Usually single dose studies are sufficient to verify all the above objectives. Regulatory agencies like the Food and Drug Administration however, may require multiple dose steady state studies for registration of the product (*Skelly*, 1986).

In-vivo evaluations based on blood level data alone may not be sufficient to optimise dosage form development. Such an approach does not allow for the evaluation of the influence of physiological variables such as gastric emptying and intestinal transit (*Digenis*, 1982), nor do these studies provide information on the integrity, distribution, and time and site of *in-vivo* disintegration and dissolution of the dosage forms (*Davis*, 1983). Moreover, these studies are not applicable to drugs that have local actions, or which are absorbed at specific sites in the gut (*Digenis*, 1982). Thus, direct visualization of the *in-vivo* behaviour of dosage forms within the gastrointestinal tract has become an important research goal in the development of modern therapeutic systems (*Wilson and Washington*, 1988).

1.2 METHODS TO STUDY *IN-VIVO* GASTROINTESTINAL TRANSIT PROPERTIES

Some of the early methods of studying gastrointestinal transit behavior of dosage forms now appear bizarre and crude, because sophisticated imaging equipment was not available. Recovery of ingested materials from the faeces was practised in order to determine whole gut transit times. An assortment of substances were administered, which included glass beads (*Alvarez and Freedlander, 1924*; *Hoelzel*, 1930), knots of cotton, tomato and grape seeds, pieces of rubber, aluminium, steel, silver and gold (*Hoelzel*, 1930). In a 'yoyo' method, used to study *in-vivo* disintegration, lengths of string were attached to the tablets for periodic withdrawal from the stomach and examined for changes (*Gruber et al*, 1958; *Steinberg et al*, 1965). Induced vomiting was also performed to recover the ingested dosage form (*Steinberg et al*, 1965). Equally distressing to the volunteers was the reported use of a fiberscope or gastroscope for viewing the dosage form in the stomach (*Steinberg et al*, 1965; *Weiss et al*, 1961; *Hey et al*, 1979).

The discovery of X-rays at the turn of the century led to the first imaging technique for external monitoring of dosage forms in the gastrointestinal tract. This method was first used by Lozinski and Diver (1933) for studying the *in-vivo* disintegration of enteric coated tablets, and subsequently by other workers (*Bukey and Brew*, 1934; *Wagner et al*, 1958; *Blythe et al*, 1959; *Levy*, 1963; *Merenda and Green*, 1966). However, this has not gained much popularity because of the radiation hazards, which limit the number of images that can be taken. Data obtained are only qualitative in nature and the high-density radio-opaque materials which are incorporated into the dosage form may themselves alter the physical characteristics of the formulation (*Daly et al*, 1982). Despite these limitations, this imaging technique still continues to be used occassionally (*Cortot and Colombel*, 1984; *Dew et al*, 1982; *Stricker and Kulke*, 1981).

Bond *et al* (1975) described a method of monitoring small bowel transit time by measuring the pulmonary hydrogen excretion following ingestion of a meal containing the non-absorbable disaccharide lactulose. This compound is fermented by colonic bacteria to yield hydrogen gas which can then be detected in breath. Therefore, a rise in hydrogen

concentration in the breath signals the arrival of the head of the meal at the caecum. Using this principle, small bowel transit time of dosage forms can be estimated by co-administering lactulose. Unfortunately, lactulose is a physiological stimulant and may shorten the normal transit time (*Read et al*, 1982), and overgrowth of colonic bacteria in the small intestines may yield erroneous results. In addition, it only measures the transit time of the head of the meal and does not give reliable data regarding the entry of the bulk of the contents into the colon (*Read et al*, 1989). The use of ileostomy patients has also been reported (*Bechgaard and Ladeford*, 1978). Examination of the ileostomy bags can yield valuable information on the behavior and transit times of ingested products. However, no information on gastric emptying can be obtained and ileostomy subjects are not readily available.

Some methods described in the literature apply more specifically to gastric emptying only. Hunt and Spurrell (1951) described a 'serial test meal' which permitted quantitative measurement of gastric emptying. The method entailed passage of a nasogastric tube, instillation of a test meal into the stomach and withdrawal of the meal after a fixed time interval. By repeating the procedure on the same subject on different days and withdrawing the gastric contents at different time intervals, the rate and pattern of emptying could be calculated. This method was later modified by George (1968) to overcome the need for repeated measurements and further refined by Meeroff *et al* (1973) to aspirate the duodenal contents by means of a transpyloric tube. A major limitation of the above procedures is the requirement for gastric or gastroduodenal intubation, thus causing discomfort to the subjects. In another innovation, Benmair *et al* (1977) described a non-invasive ferromagnetic method where an inert tracer, magnesium ferrite, was added to a meal. The content in the stomach was then monitored at regular intervals using an external transducer. Two methods recently described in the literature are impedance epigastrography (*Sutton et al*, 1985) and applied potential tomography (*Avill et al*, 1987). Both methods detect the changes in gastric electrical impedance when a liquid meal empties from the stomach. Ultrasound was used by Bateman and Whittingham (1982) to measure the gastric emptying of a liquid test meal. This technique was later applied by Bolondi *et al* (1985) to measure the gastric emptying of mixed solid-liquid meals. More recently, visualization of tablets in the gastrointestinal tract using sonography was reported by Maublant *et al* (1988), but the tablets could not be detected once they had entered the intestine.

Indirect methods of measuring gastric emptying have also been described. Such methods rely on the fact that most drugs are preferentially absorbed in the small intestines. Therefore, the rate at which such a drug appears in the blood stream can be used as a measure of the gastric emptying rate. This has been demonstrated with paracetamol (*Heading et al*, 1973; *Clements et al*, 1978) and ethanol (*Finch et al*, 1974). Indirect measure of the orocaecal transit time was also reported using salazopyrin (*Kennedy et al*, 1979). This compound is hydrolysed to sulphapyridine and 5-aminosalicylic acid in the large bowel. Measurement of sulphapyridine in the plasma was used to determine the orocaecal transit time.

Application of radionuclides in gastrointestinal studies was first used by Hansky and Connell (1962). Total transit times were measured by recording the activity of Chromium-51 labelled compounds excreted in the faeces after oral administration. This was later refined to monitor gastric emptying by scanning the stomach region for loss of counts using a scintiscanner (*Griffith et al*, 1966). Improvement in measuring the radioactivity was made when Harvey *et al* (1970) and Jones *et al* (1970) reported using a gamma camera in gastric emptying studies with the radionuclides Chromium-51 and Caesium-129 respectively. The gamma-camera enabled the whole region of interest to be viewed continuously and produced frequent images of the distribution of radioactivity. This development together with the discovery of almost ideal radionuclides 99m-Tc, ^{111}In and ^{113m}In have contributed to the subsequent growth in the use of this method (*Davis*, 1983). Ever since its applications to pharmaceuticals were reported by Casey *et al* (1976) and Alpsten *et al* (1976), this imaging technique has superceded all the other methods in gastrointestinal transit studies of dosage forms. (*Fell and Digenis*, 1984).

Gamma scintigraphy, as this imaging technique is termed, represents the first method that allows complete characterization of the gastric emptying and intestinal transit processes. It is non-invasive, only exposes the subject to low radiation doses, and provides information on a continuous and quantitative basis. Neutron activation techniques can be used to overcome some of the problems encountered in labelling of the dosage forms (*Christensen et al*, 1984; *Parr et al*, 1985). The location and *in-vivo* dissolution of certain dosage forms can also be monitored using suitable radiolabelled model compounds that can mimic the release of the drug of interest (*Beihn and Digenis*, 1981; *Sournac et al*, 1988; *Davis et al*, 1989). A further

advantage of the technique is its ability to monitor two isotopes which have been added to the ingested contents simultaneously and independently.

1.3 THEOPHYLLINE

1.3.1 GENERAL PHARMACOLOGY

Theophylline is a methylated xanthine alkaloid (1,3-dimethylxanthine) found in plants and is very similar in structure to the other naturally occurring xanthines, caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine). The principal therapeutic use of theophylline is as a bronchodilator (*May*, 1974). However, its spectrum of pharmacological activity in treating asthma, includes not only relief of acute symptoms due to its relaxant effect on bronchial smooth muscle but also suppression of chronic symptoms of airway hyper-reactivity and inhibition of exercise induced broncho-constriction (*Hendeles and Weinberger*, 1983). In recent years, therapeutic use of theophylline has not been confined to patients with reversible airway disease. There is substantial interest in its use for treating patients with relatively fixed airway obstruction. The action of theophylline in this latter group is due to its ability to improve myocardial contractility (*Matthay et al*, 1978) diaphragmatic and intercostal muscle contractility, as well as delaying the onset of fatigue (*Aubier et al*, 1981). It is also widely used in the treatment of apnoea in premature infants because of its stimulatory activity on the respiratory centre (*Dietrich et al*, 1978).

The mechanism of action of theophylline is not clearly established. Inhibition of cyclic AMP phosphodiesterase activity was proposed, but concentrations required to demonstrate this property *in-vitro*, were not attainable *in-vivo* due to toxicity (*Svedmyr*, 1977). Moreover, other potent phosphodiesterase inhibitors (example papavarine) were shown to be ineffective in asthma (*Bergstrand*, 1980). However, theophylline is known to affect calcium flux across cell membranes (*Brisson et al*, 1972), to act as a prostaglandin antagonist (*Horrobin et al*, 1977), as an antagonist of adenosine (*Fredholm*, 1980) and also to cause increased binding of cyclic AMP to cyclic AMP binding proteins (*Miech et al*, 1979).

The pharmacodynamic effects of theophylline in relation to its serum concentration are well defined. In the treatment of apnoea in premature infants, serum concentrations in the range of 5 to 10 mcg/ml were found effective in decreasing the number of apnoeic episodes (*Dietrich et al*, 1978). The bronchodilator effect was reported to increase with the serum concentration over a range of 5 to 20 mcg/ml (*Mitenko and Ogilvie*, 1973). However, a

theophylline serum concentration of between 10 and 20 mcg/ml has received general acceptance as providing optimal control of asthmatic symptoms (*Weinberger and Bronsky*, 1974). Whilst additional bronchodilator effects may be achieved with a serum concentration greater than 20 mcg/ml, this is usually associated with an increasing frequency of adverse effects, which include nausea, vomiting, diarrhoea, headache, and irritability (*Zwillich et al*, 1975; *Jacobs et al* , 1976; *Hendeles et al*, 1977). Serum concentrations exceeding 35 mcg/ml have been associated with life threatening cardiac arrhythmias, seizures and death (*Hendeles et al*, 1977).

Theophylline solution is rapidly and completely absorbed throughout the length of the gastrointestinal tract, but the rate of absorption in the colon is reduced when compared to the upper gastrointestinal tract (*Staib et al*, 1986). Once absorbed, it distributes rapidly into tissues and distribution is complete within 30 to 45 minutes. Thus kinetic analysis can be satisfactorily applied using a one compartment pharmacokinetic model (*Mungall*, 1983; *Loughnan et al*, 1976). It is extensively metabolized in the liver to the major metabolites 1-methyluric acid and 1,3-dimethyluric acid and other minor methylxanthine metabolites which are excreted in the urine (*Jenne et al*, 1976). Less than 10% of the drug is excreted in the urine unchanged (*Levy and Koysooko*, 1976). Nonlinearity of elimination resulting from saturation of some of the metabolic pathways has been reported to occur in certain susceptible individuals (*Weinberger and Ginchansky*, 1977; *Tang-Liu et al*, 1982; *Gundert-Remy et al*, 1983). On the other hand, dose-ranging studies in normal adults (*Koeter et al*, 1981; *Rovei et al*, 1982) and in adult asthmatic patients (*Brown et al*, 1983) have shown that elimination of theophylline followed first-order kinetics. Studies by Upton *et al* (1980a, 1980b) also suggested that the elimination is linear within the therapeutic range . Other studies have shown that dose-dependent elimination is more frequently encountered in children, but for the majority of adults, theophylline kinetics can be described adequately using a linear model (*Mungall*, 1983).

Clearance of theophylline can be modified by a variety of factors. Mean plasma clearance rates are markedly reduced in neonates (*Aranda et al*, 1976), and infants only gradually approach normal childhood values over the first eight months of life (*Nassif et al*, 1981). Physiological abnormalities such as cardiac disease (*Hepner and Vessel*, 1978), hepatic dysfunction (*Mangione et al*, 1978), acute viral illness (*Chang et al*, 1978), and pneumonia

(*Voseh et al*, 1978) have all been reported to decrease theophylline clearance. Concurrent administration of some drugs such as erythromycin (*Kozak et al*, 1977), mexiletine (*Loi and Vestal*, 1990), tetracycline (*McComack*, 1990), and cimetidine (*Bauman and Kimelbatt* 1982; *Krstenansky et al*, 1989) also appears to decrease theophylline clearance. In contrast, cigarette smoking (*Jusko et al*, 1978), high protein, low carbohydrate diet (*Kappas et al*, 1976), charcoal broiled meats (*Kappas et al*, 1978) and enzyme-inducing drugs such as phenytoin (*Crowley et al*, 1987) and rifampicin (*Miners and Wing*, 1984) have been found to increase the rate of theophylline elimination. It is interesting to note that passive smokers, that is non-smokers with long term exposure to cigarette smoke, have been reported to show an increased rate of elimination of theophylline (*Matsunga et al*, 1989).

1.3.2 SUSTAINED RELEASE THEOPHYLLINE FORMULATIONS

Theophylline has been used in the treatment of reversible airway disease since the early 1930s, but its use declined during the 1960s following reports of acute toxicity and death in children treated with the conventional drug formulations (*Mungall*, 1983). In the 1970s pharmacodynamic and pharmacokinetic studies gave a better understanding of its dose related behaviour. Sustained release formulations of theophylline were first introduced in the early 1970s, and the reduced fluctuation of serum concentrations and decreased frequency of administration provided by these preparations, greatly enhanced their safe and efficacious use in chronic therapy (*Shangraw* 1988).

The perception of a large market has resulted in the development of a considerable range of sustained release theophylline products. An excellent review on their design and formulation was recently published (*Shangraw*, 1988). In general, the sustained release mechanisms of these products can be reduced to three basic types, namely, erosion of a matrix (embedded with the drug), erosion of a coat surrounding a drug core or diffusion of the drug through a nonsoluble coat. Materials which have been used to form the erodible matrix or coat include lipid substances that are eroded by mechanisms such as emulsification by bile salts or lipase hydrolysis; acidic polymers whose solubilities are pH dependent; and water soluble polymers that swell and gradually dissolve. In the case of the non-soluble coat the main coating material used is ethylcellulose. Some of these products are marketed as single unit tablets, whereas others are in the form of multi-unit pellets contained in a capsule. In certain formulations, the single unit tablet may disintegrate into multiple pellets (with sustained

release properties) upon exposure to the dissolution medium. The early sustained release theophylline products were mainly designed for a twice daily dosing, but in recent years, once-daily preparations have become available.

Certain sustained release theophylline products were shown to exhibit incomplete and erratic absorption (*Boroda et al*, 1973; *Weinberger et al*, 1978; *Hurwitz et al*, 1987). Whilst such erratic performance might be an inherent fault of the formulation itself, food, particularly that with high fat content, has been reported to affect the absorption of some of these preparations. A number of studies have shown that the effects of food are very variable. Both the rate and extent of absorption of the commercial preparation 'Theo-Dur Sprinkle' (Key Pharmaceuticals Inc., US) were considerably reduced when taken with food (*Pedersen and Moller-Petersen*, 1984; *Karim et al*, 1985). Similar decreases in bioavailability were also reported with 'Theolong' (Eisai Co. Ltd., Tokyo) and 'Theodur-G' (Mitsubishi Chemical Industry Co. Ltd., Tokyo): the effect was more pronounced with fat-rich diets (*Tada et al*, 1989). Some products however, only exhibited a delayed or decreased rate of absorption, while the amount absorbed remained unchanged when administered with food. This has been shown with 'Theo-Dur' - Key Pharmaceuticals Inc. US (*Leeds et al*, 1982; *Osman et al*, 1983; *Spector*, 1985, 1986; *Spector et al*, 1985), 'Slo-Bid Gyrocaps' - Rorer Pharmaceuticals, US (*Hendeles and Weinberger*, 1986), 'Theolair SR' - Riker Laboratories Inc. USA (*Pedersen*, 1981; *Pedersen and Moller-Petersen*, 1982) and 'Somophyllin CRT' - Fisons Corporation US (*Pedersen and Moller-Petersen*, 1985). Finally, with 'Theograd' (Abbott Laboratories, UK), although food appeared to decrease the rate of absorption, the total amount absorbed was increased (*Lagas and Jonkman*, 1983, 1985).

A more serious consequence of food effect occurs if both the rate and extent of absorption are increased due to dose-dumping, especially if the entire 24 hour dose is delivered in a once-daily formulation. Peak serum concentration of the once-daily 'Uniphyl' (Purdue Frederick Co. USA) was increased by almost 100% when the product was taken with a high fat diet, but the time to peak was also almost doubled (*Karim et al*, 1985). Similar food effects were confirmed by Milavetz *et al*, (1986) with this product. Furthermore, serious dose-dumping was reported by Hendeles *et al* (1985) with the product 'Theo-24' (G D Searle and Co., US), and in another study with 'Uniphyllin' (Mundipharma, Federal Republic of

Germany) in paediatric patients, dose-dumping was observed in 3 out of 8 children tested, resulting in toxic serum concentrations (*Steffensen and Pedersen*, 1986).

There are a number of products which have been reported to be relatively unaffected by food. Examples of this are 'Euphylong' - Byk Gulden Pharmaceuticals, Federal Republic of Germany (*Schulz et al*, 1987), 'Dilatrane AP' - Fisons plc, UK (*Delhotal-Landes et al*, 1988), 'Sabidal' - Zyma SA, Switzerland (*Jonkman et al*, 1985) and 'Theobid Duracap' - Glaxo, US (*Osman et al*, 1983). The literature reviewed above is by no means exhaustive, but it serves to illustrate the potential of variable food interaction with sustained release products of theophylline. Not all brands currently used in daily practice have been studied in this respect (*Jonkman*, 1989), and therefore their performance under different food status are not known.

Schulz *et al* (1987) investigated the effects of hot and cold meals. They found that a hot evening meal tended to decrease the bioavailability when compared to a cold one. The influence of *in-vivo* pH was also studied by Myhre and Walstad (1983) who co-administered an antacid with the theophylline preparations. Co-administration of antacid was found to increase the serum theophylline levels of 'Neulin Depot' (Riker Laboratories Ltd., England), but the serum levels of 'Theo-Dur' (Draco A B, Sweden) were not significantly affected. *In-vitro* tests showed that whilst the drug release of 'Neulin Depot' was pH dependent, that of 'Theo-Dur' was not. In another study by Vashi and Meyer (1988), absorption of 'Theo-24' (Searle and Co., Skokie, Il) was found to increase in dogs treated with ranitidine. In contrast, the other preparation tested, 'Labid' (Norwich Eaton Inc., Norwich, NY) exhibited decreased absorption of theophylline when ranitidine was given concomitantly. Both preparations however, showed pH dependent dissolution.

More recently, the effect of altering the small bowel transit time on the absorption of 'Theo-24' (G D Searle & Co., US) was investigated by concurrent administration of metoclopramide and loperamide to decrease and increase the transit time respectively (*Bryson et al*, 1989). Small bowel transit time was measured using the hydrogen breath technique described by *Bond et al*, (1975). Metoclopramide had no effect on both the rate and extent of absorption. In contrast, loperamide decreased the rate but not the extent of absorption. In another study, Sommers *et al*, (1990) monitored the orocaecal transit time of 'Theo-Dur' by co-administration of sulphalazine. The medication reached the large bowel at about 5.4 hours

and at least 38% of the administered dose was additionally absorbed over the next 25 hours in the large intestine. Soumac *et al* (1988) also monitored the gastrointestinal transit and *in-vivo* release of a sustained release theophylline tablet ('Theostat 300 mg' Laboratoires Sinbio, Paris, France) by incorporating [99m-Tc] DTPA (diethylenetriamine penta-acetic acid) into the tablet formulation. The *in-vivo* release kinetics of theophylline was calculated from plasma level data, while the [99m-Tc] DTPA release was determined using gamma scintigraphy. Satisfactory agreement was observed between theophylline and [99m-Tc] DTPA release rates, both *in-vivo* and *in-vitro*. Davis *et al* (1989) conducted a similar study on Phyllocontin Continus Tablet (Napp Laboratories UK) which contained the ethylenediamine salt of theophylline (aminophylline). A different radiolabelled marker which better mimicked the aminophylline release was used. These workers also reported good agreement between *in-vitro* and *in-vivo* release rates, and that the *in-vivo* release was independent of changing pH and degree of gastrointestinal motility.

1.4 EXPERIMENTAL AND SCOPE OF THE PRESENT STUDY

As discussed in section 1.1.3, a multiunit dosage form appears to offer a number of advantages over the single unit system in drug therapy with sustained release formulations. On the other hand, a drug such as theophylline (section 1.3), which is known to be well absorbed throughout the length of the gastrointestinal tract, and which has a narrow therapeutic index, represents an ideal candidate for this form of drug delivery. In view of all these, the present study was undertaken to develop a multiunit sustained release preparation of theophylline, and to evaluate its *in-vivo* performance as well as its gastrointestinal transit properties.

The study was conducted in various stages with the following objectives:

1) To prepare spherical theophylline pellets of high drug loading
2) To develop a suitable film coat for coating the pellets to effect sustained drug release
3) To evaluate the *in-vivo* release and extent of absorption in healthy human volunteers when a satisfactory *in-vitro* release profile was achieved, and also to interrelate *in-vitro* and *in-vivo* results
4) To compare bioavailability of the novel preparation with a commercial sustained release theophylline product under fed and fasted conditions using healthy human volunteers
5) To determine the gastrointestinal transit properties of the novel preparation under fed and fasted conditions
6) To relate the drug absorption with gastrointestinal transit properties.

EXPERIMENTAL

Chapter 2: PREPARATION OF THEOPHYLLINE PELLETS

2.1 INTRODUCTION

Spherical pellets were prepared using an extrusion and spheronisation process described by Reynolds (1970) and Conine and Hadley (1970). In this technique, the medicaments and excipients are initially mixed with suitable binders and water to form a wet plastic mass. This is followed by a preforming stage, in which the mass is extruded and shaped into cylindrical strands of uniform diameter. When this is satisfactorily achieved, the extrudates are broken into short equal lengths and rolled into spheres.

There are several types of extruders but, essentially, they all provide the same mechanism of forced feeding the wet mixture through a die to form the cylindrical shaped segments. On the other hand, the spheroniser consists of a grooved horizontal plate rotated within a stationary vertical cylinder fitted with a door to allow discharge of the rounded products. The speed of rotation can be varied and the geometry of the grooves altered by changing the rotating plate.

When the extrudates are loaded into the spheroniser, they are initially broken down into short lengths by the rotating plate, and transported by the centrifugal force to the periphery of the spheroniser. Their residual momentum causes them to rise up the stationary wall in a circular motion and then fall as this momentum is dissipated. The interaction with the plate as well as the interparticulate friction created as the pellets roll over each other, cause the broken extrudates to go through a sequence of shape changes before achieving a spherical shape (*Reynolds*, 1970). The ends of the cylindrical segments are first rounded before forming 'dumb-bell' shapes, then ellipsoids and finally spheroids.

The composition of the wet mass and hence its rheological properties are crucial to the success of both the extrusion and spheronisation processes. The wet mass should be sufficiently plastic not only to undergo the deformation when it passes though the die during extrusion, but also to be rounded when spheronised. At the same time however, the mixture is required to produce extrudates that are adequately brittle, so that they can be broken into uniform lengths in the spheroniser. A mixture that is not sufficiently plastic may in the first

instance be difficult to extrude. Even if extrusion can be achieved, a spherical product is not obtainable but remains cylindrical or ellipsoidal. On the other hand, if the extrudates are plastic enough to deform but insufficiently brittle, a mean size of spheres larger than desired is obtained (*Conine and Hadley*, 1970). Usually, microcrystalline cellulose is used as an excipient because it exhibits the elasticity required for extrusion and spheronisation (*Miyake et al*, 1973). However, the moisture content is also highly critical in ensuring a mass of sufficient plasticity. Too much moisture on the other hand may cause the extrudates to agglomerate uncontrollably during spheronisation (*Gamlen*, 1985).

In addition to the composition of the wet mass, the die geometry and speed of extrusion are also important parameters to be considered for obtaining a good extrusion product (*Harrison et al*, 1985). It was found that improper extrusion conditions may lead to physical defects in the extrudates resulting in a poor spheronised product. Other factors which are important in the spheronisation include the quantity processed, geometry of the plate and speed of rotation (*Reynolds*, 1970). Chapman (1985) showed that these parameters could be optimised to produce a good product.

The aim of this part of the study was to prepare spherical theophylline pellets of high drug loading and rapid rate of dissolution. Several formulations were evaluated for their suitability in preparing spherical pellets using the extrusion/spheronisation method. The theophylline content was progressively increased to a maximum value consistent with the successful preparation of spherical pellets. In addition, Amberlite pellets of similar size and density were also prepared. They were to be used for monitoring the gastrointestinal transit properties of the theophylline pellets in a later study.

2.2 MATERIALS AND METHODS

2.2.1 MATERIALS

Avicel PH101, BH0747 FMC Corporation, Pennsylvania, USA

Amberlite® CG-400 ion-exchange resins, 100-200 wet mesh, # 22429, Aldrich Chemical Co. Ltd. England

Lactose Fine 170, Dairy Crest, Surrey

Anhydrous theophylline BP, BASF, United Kingdom Ltd., Lot number 58786

2.2.2 METHOD OF PREPARATION

The formulae used in the preparation of the pellets are as shown in Table 1. The dry powders were blended in a Kenwood planetary mixer (Model A707A, Havant, Harts) for 5 minutes. Sufficient deionized water was added and mixing continued for another 10 minutes. In the case of the 5% Amberlite formula, the Amberlite powder was initially mixed with Avicel following a geometric dilution procedure prior to incorporation of the lactose powder. The final mixture was stored in a polyethylene bag until use to prevent loss of moisture.

Extrusion of the wet powder mass was carried out using a ram extruder (*Ovenston and Benbow*, 1968). The material was packed into the barrel (2.54 cm internal diameter and 20.3 cm length) of the ram extruder fitted with single holed die of hole size 1 mm diameter and 4 mm length. The piston was inserted into the barrel to partially consolidate the mass inside. When sufficient material has been packed, the whole assembly was mounted on a C-piece support and aligned underneath the crosshead of a Llyods press (MX50, Llyods Instruments). Extrusion was then carried out with the crosshead moving down at a constant displacement rate of 20-40 cm/min and the force displacement profile was recorded on a computer. The products of the extrusion were collected and 200 - 300 G loaded into a 8.5 inch spheroniser (G B Caleva, Ascot, Berks) with a radially grooved plate rotated at 1000 rpm for periods of 15 - 30 minutes. At the end of the spheronisation, the pellets were collected and dried in a fluid bed drier (P R L Engineering, Flintshire), at 60 °C for 1 hour. The dried spheres were sieved with mesh sizes of 1.0, 1.18, 1.4 and 1.7 mm using a mechanical sieve shaker (Endecotts, London) for 15 minutes. Only the 1.18 - 1.4 mm fraction of spheres were used in further studies.

Table 1 : FORMULAE OF THEOPHYLLINE AND 5% AMBERLITE PELLETS

Ingredients	Percentage of theophylline			
	66.7	75.0	80.0	0.0
theophylline	300 G	300 G	300 G	-
Avicel PH101	200 G	100 G	56.3 G	135 G
water	320 G	218 G	160 G	170 G
lactose	-	-	18.7 G	150 G
Amberlite	-	-	-	15 G

2.2.3 *IN-VITRO* DISSOLUTION STUDIES

The *in-vitro* theophylline release of the uncoated pellets was determined using the paddle unit (method 2) of the USP XXI dissolution test apparatus (model PTWS, Pharma Test Apparatebau, W Germany). All the tests were conducted in 900 ml of dissolution medium maintained at 37.0 $\pm$ 0.5 °C with a paddle rotation speed of 100 rpm. In each case, the weight of theophylline pellets used was 300 mg. Samples of 3 ml volume were withdrawn at various predetermined time intervals using an automated sampler (Pharma Test Apparatebau Type PTFC1, W Germany). The drug concentration of the samples was determined by direct measurement of the UV absorbance at 273nm using a Perkin-Elmer 554 UV-Vis. spectrophotometer after appropriate dilution. Preliminary experiments have established a linear relationship between drug concentrations and absorbance values. Each test was run in sets of six and the average percentage released over time was then calculated. Different dissolution media were used and they included 0.1N hydrochloric acid, and phosphate buffer BP of pH 4 and pH 7. To study the stability of theophylline release with storage times, some of the experiments were repeated six months and twelve months later.

2.3 RESULTS AND DISCUSSION

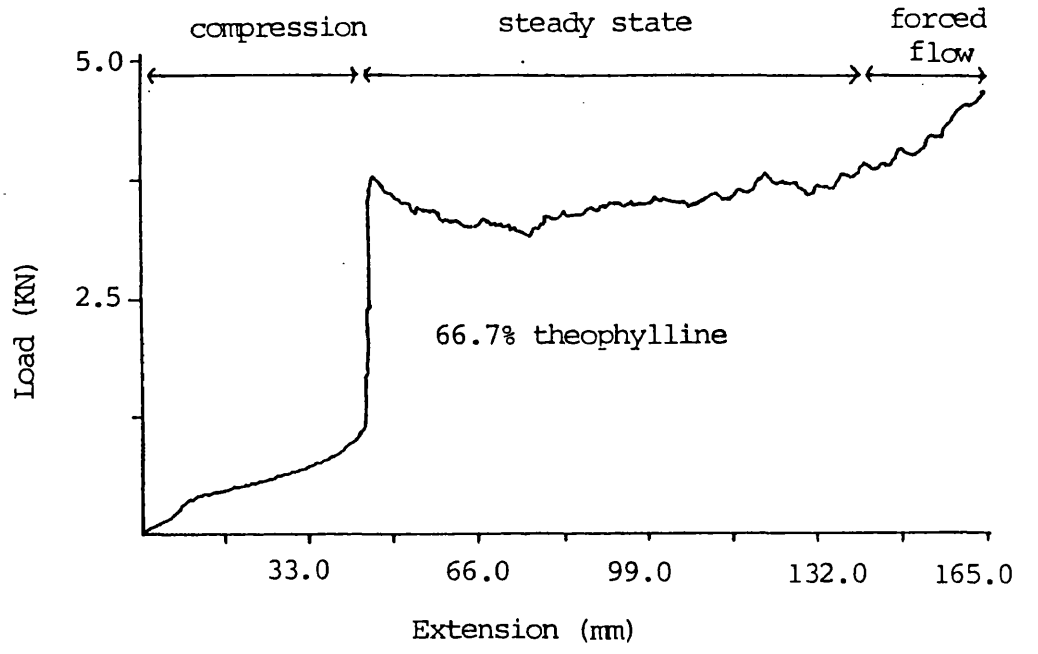
2.3.1 EXTRUSION AND SPHERONISATION

Spherical pellets were successfully prepared from the formulae shown in Table 1. The extrudates obtained were smooth and cylindrical with no visible defects such as 'shark-skinning' (*Harrison et al*, 1985) and when spheronised, the pellets were adequately rounded. Figures 1 and 2 show the typical force/displacement profiles produced during the extrusion process. Three distinct regions are clearly visible from all the plots. They correspond to a compression stage, a steady state flow stage and a forced flow stage. The interpretation and the factors affecting the force/displacement profile at the various stages of extrusion have been well discussed by Harrison *et al* (1985). They explained that during the compression stage, the material which was initially hand packed in the barrel, was consolidated, requiring only a slight application of force. Once this was complete, there was a sharp rise in the ram force, marking the start of the steady state flow. At this stage, both the ram force and flow pattern of the material in the barrel remained fairly constant. In the forced flow stage, an increasing ram force was required to maintain the flow and it also coincided with a change in flow pattern of the wet mass.

Harrison *et al* (1985) found that the materials left behind in the barrel during forced flow, were of lower moisture content when compared to the extrudates, and this phenomenon was less likely to occur at faster ram speeds. Therefore, a relatively long forced flow stage is undesirable and should be minimized, as it may affect the quality and homogeneity of the extrudates. The forced flow stage obtained with all the formulae shown in Table 1, was relatively brief and could be further minimized, either by increasing of ram speed from 20 to 40 cm/min or by shortening the length of displacement of the piston in the barrel.

Whilst no attempt was made to study the process variables in detail, a few important specific points relevant to this particular process were apparent. As the concentration of theophylline was increased, both the extrusion and spheronisation of the mixture became more difficult to control, and were critically dependent on the moisture content. The problem was more acute when the concentration of theophylline was greater than 80%. Insufficient moisture resulted in early onset of forced flow during extrusion, and in more extreme cases, there was early

Figure 1 : FORCE/DISPLACEMENT PROFILES OF 66.7% AND 75.0% THEOPHYLLINE FORMULAE



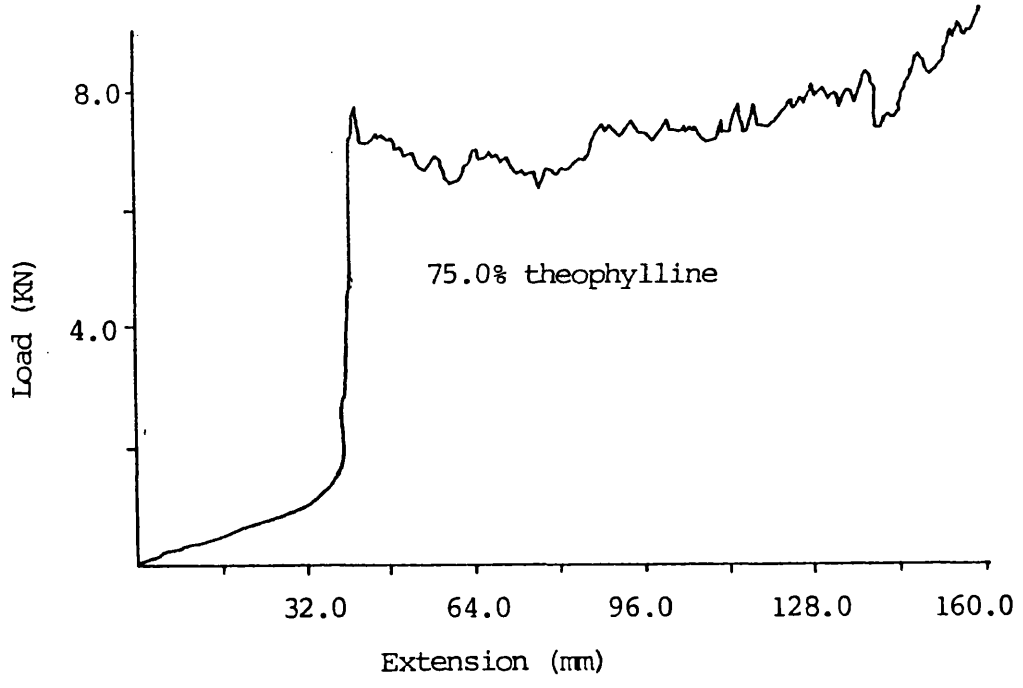
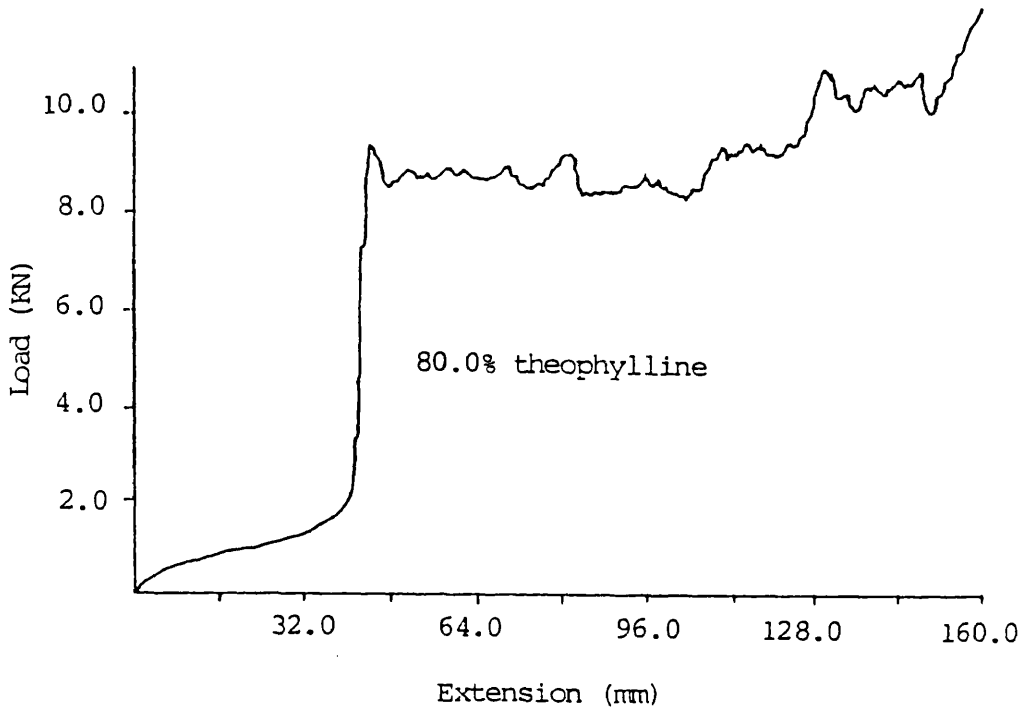
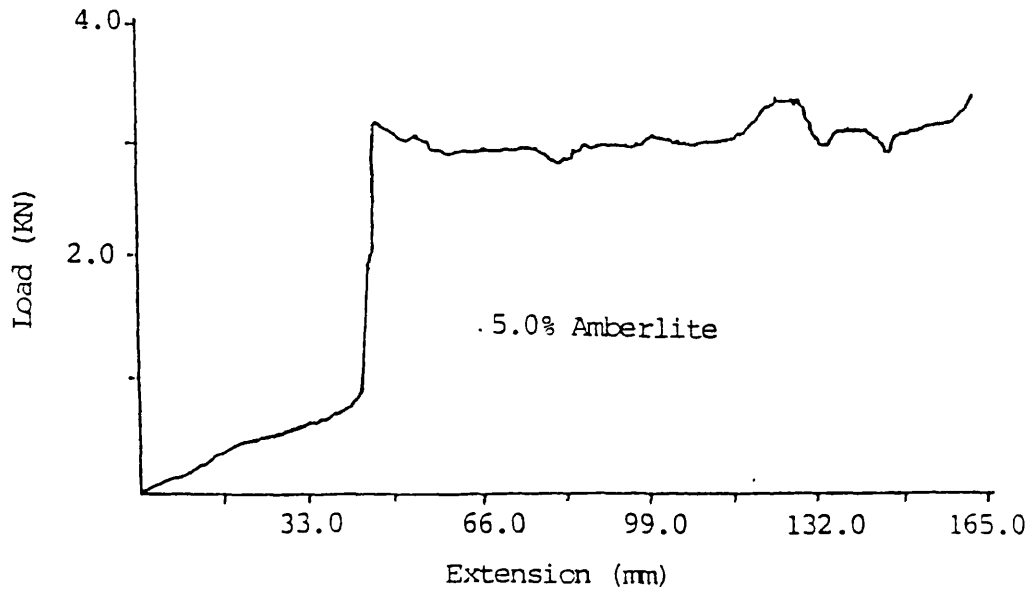


Figure 2 : FORCE/DISPLACEMENT PROFILES OF 80.0% THEOPHYLLINE AND 5.0% AMBERLITE FORMULAE





cessation of flow as the ram force rose rapidly above the preset maximum value. Under these conditions, the extrudates produced were often insufficiently plastic and could not be satisfactorily spheronised into rounded spheres. Usually a mixture of ellipsoids and rounded end cylinders was produced. At best, a mixture containing only a low proportion of adequately rounded pellets was obtained. Generally, however, increasing the water content and ram speed could overcome the extrusion problems. The overall force/displacement profile became more constant with a shortened forced flow period and the products of extrusion were smooth and cylindrical. However, in spite of the good extrusion characteristics, the extrudates did not spheronise well. The pellets tended to stick to one another and agglomerate into large pellets. Further manipulations of the water content were usually of no avail, and a satisfactory product could not be prepared with a percentage of theophylline greater than 80%.

In view of all the processing problems, the maximum amount of theophylline incorporated into the formulations was limited to 80%. At this percentage, a satisfactory product could be formed by careful adjustment of the water content and a longer spheronisation time. When the theophylline concentration was 66.7%, spheronisation was complete in 15 minutes. But when the concentration was increased to 75 and 80%, spheronisation had to be continued for up to 30 minutes before a satisfactory product was formed. Since the Avicel component is responsible for the deformable properties of the mixture, a reduction in these properties at high drug concentrations has to be compensated with a longer spheronisation time.

The size distribution of the pellets is shown in Table 2. It can be inferred that, at higher theophylline concentrations, there is an increase in the fraction of the larger size pellets. The most obvious change is in the fraction of pellets between the size range of 1.0 - 1.18 mm and 1.4 - 1.7 mm, while the fraction in the size range of 1.18 - 1.4 mm remains relatively unchanged. These findings are consistent with the results of Clarke (1989), who reported an increase in the mean size of barium sulphate pellets when the concentration was high. The increase in pellet size may be attributed to the extrudates being less brittle at high drug concentrations, and the addition of 5% lactose into the 80% theophylline formulation was aimed at circumventing this problem. In all the formulations the yield of pellets in the size range of interest (1.18 - 1.4 mm) was more than 60% and was deemed satisfactory for the present study.

Table 2 : SIZE DISTRIBUTION OF THEOPHYLLINE AND 5% AMBERLITE PELLETS

Size range (mm)	66.7% theophylline	75.0% theophylline	80.0% theophylline	5.0% Amberlite
> 1.7	-	0.1(0.05)	0.4(0.1)	-
1.4 - 1.7	0.7(0.4)	24.1(7.6)	33.1(2.8)	1.0(0.2)
1.18 - 1.4	74.0(5.0)	69.8(6.7)	62.1(4.2)	76.1(3.8)
1.0 - 1.18	24.2(4.7)	4.9(0.5)	3.9(1.1)	22.0(3.3)
< 1.0	1.1(0.4)	1.1(0.4)	0.5(0.3)	0.9(0.4)

. Figures in brackets = standard deviation (n=3)

2.3.2 *IN-VITRO* THEOPHYLLINE RELEASE

The dissolution results of the theophylline pellets prepared using the three formulae are shown in Figures 3-5. Drug release from all three types of pellets appears relatively unaffected by pH, and is fairly rapid with almost complete dissolution within 4 hours. Similar lack of pH dependency of drug release was observed by Biswanath *et al* (1990) with theophylline micropellets in pH 1 and pH 7.4. A comparison of the dissolution profiles in Figure 6 reveals that the rate of drug release is relatively faster with pellets of higher drug content. A possible explanation for the increase in rate of drug release is that there are more drug particles per pellet and consequently, the effective surface area exposed to the dissolution medium is greater, compared to those of lower drug concentrations. Also, it was observed that the pellets were non-disintegrating, but remained intact during the course of dissolution. As such, the undissolved part of the pellets, comprising microcrystalline cellulose, can behave like an inert matrix system. Pellets with a higher drug to polymer ratio will yield a matrix that is more porous in nature, with lesser retarding effect on the drug dissolution and hence is faster.

It has been reported by Zhang *et al* (1990) that the drug release from their acetaminophen pellets occurred through this matrix mechanism. These pellets were also prepared using the extrusion/spheronisation method with Avicel as the sole excipient. They demonstrated that the acetaminophen dissolution profiles obeyed the square root law proposed by Higuchi (1963) for diffusion controlled release from such inert matrix systems. In another study with ethylcellulose micropellets, Biswanath *et al* (1990) also reported that the theophylline release was governed by this mechanism. However, when the dissolution profiles of the pellets in the present study were analyzed according to this diffusion model, poor fits were obtained. Instead, they were better fitted using a first order plot as shown in Figure 7. Except for an initial curvature, which can be attributed to the presence of drug on the surface of the pellets, a linear relationship is obtained with all the plots. It is apparent therefore, that the drug release may not be fully dependent on the matrix system, but other mechanisms may be operative. The difference in the dissolution profiles compared to those reported by Zhang *et al* (1990) and Biswanath *et al* (1990) may be ascribed to the higher polymer to drug ratio utilized by these workers. A ratio of 1 was used in some of their formulations, and with the high polymer content, a compact structure more befitting of a true matrix system can be formed. This may explain the goodness of fit obtained by their formulations with the square root law.

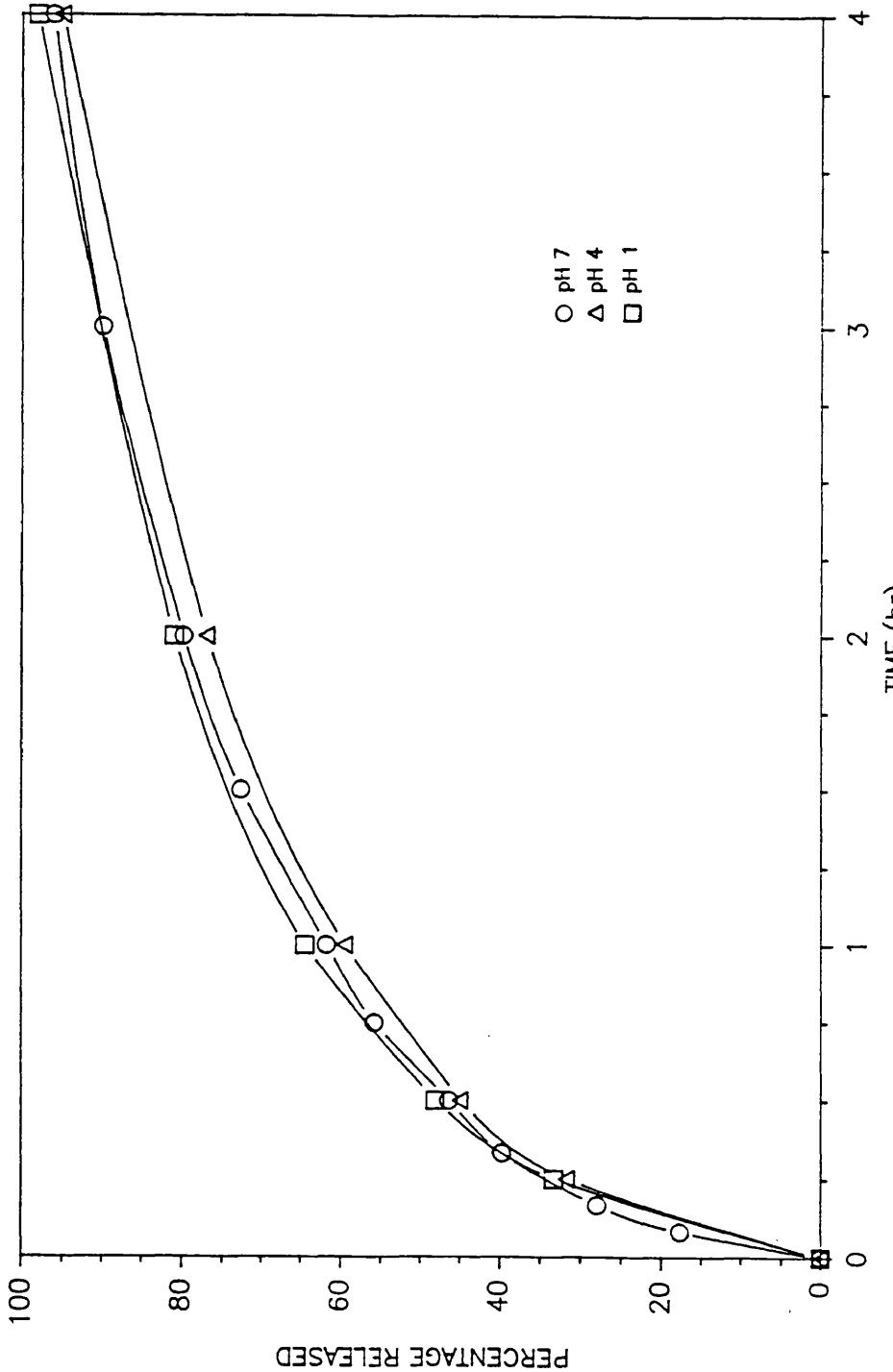


FIGURE 3 : IN-VITRO RELEASE FROM UNCOATED 66.7% THEOPHYLLINE PELLETS AS A FUNCTION OF pH

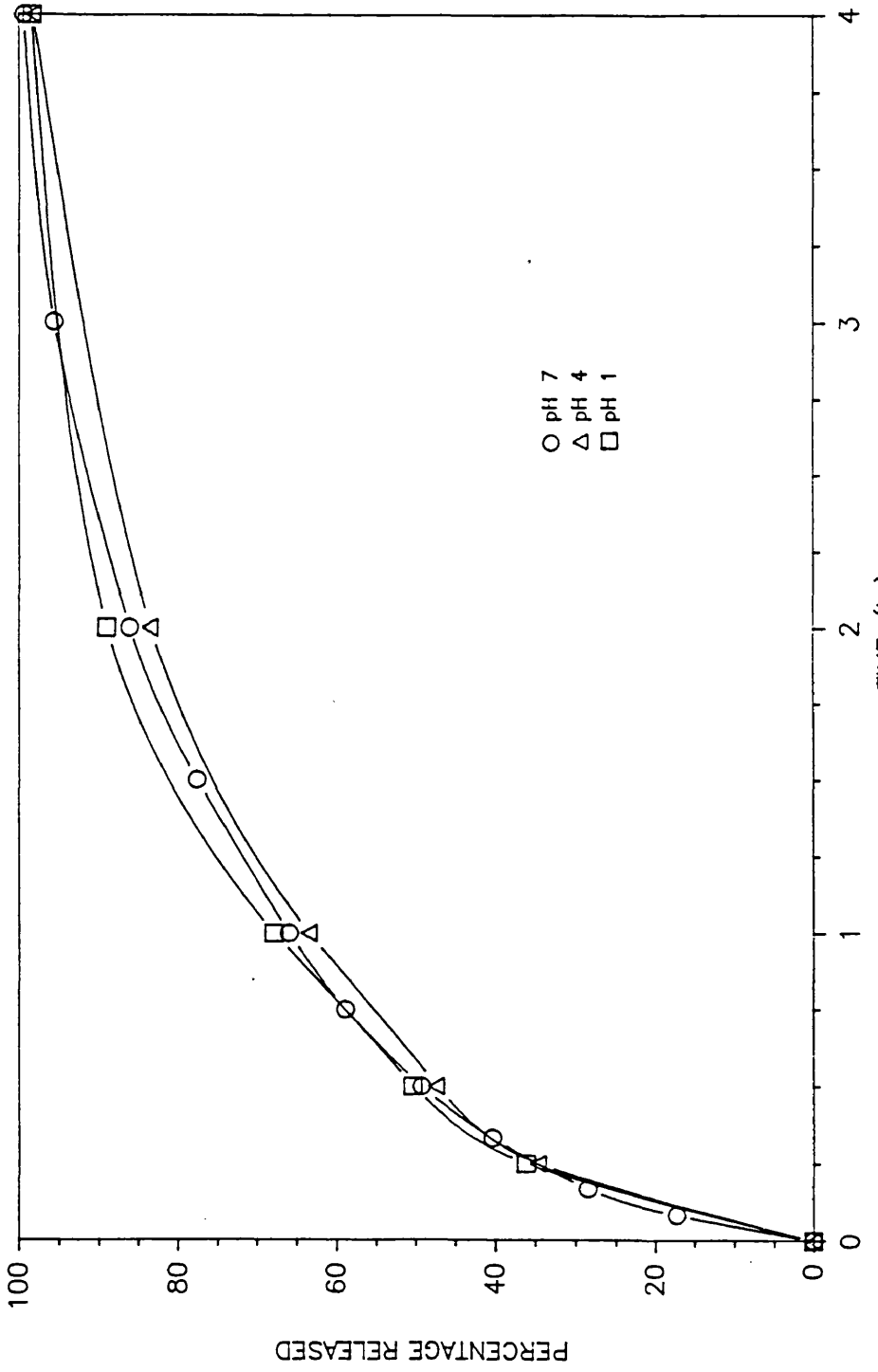


FIGURE 4 : IN-VITRO RELEASE FROM UNCOATED 75% THEOPHYLLINE PELLETS AS A FUNCTION OF pH

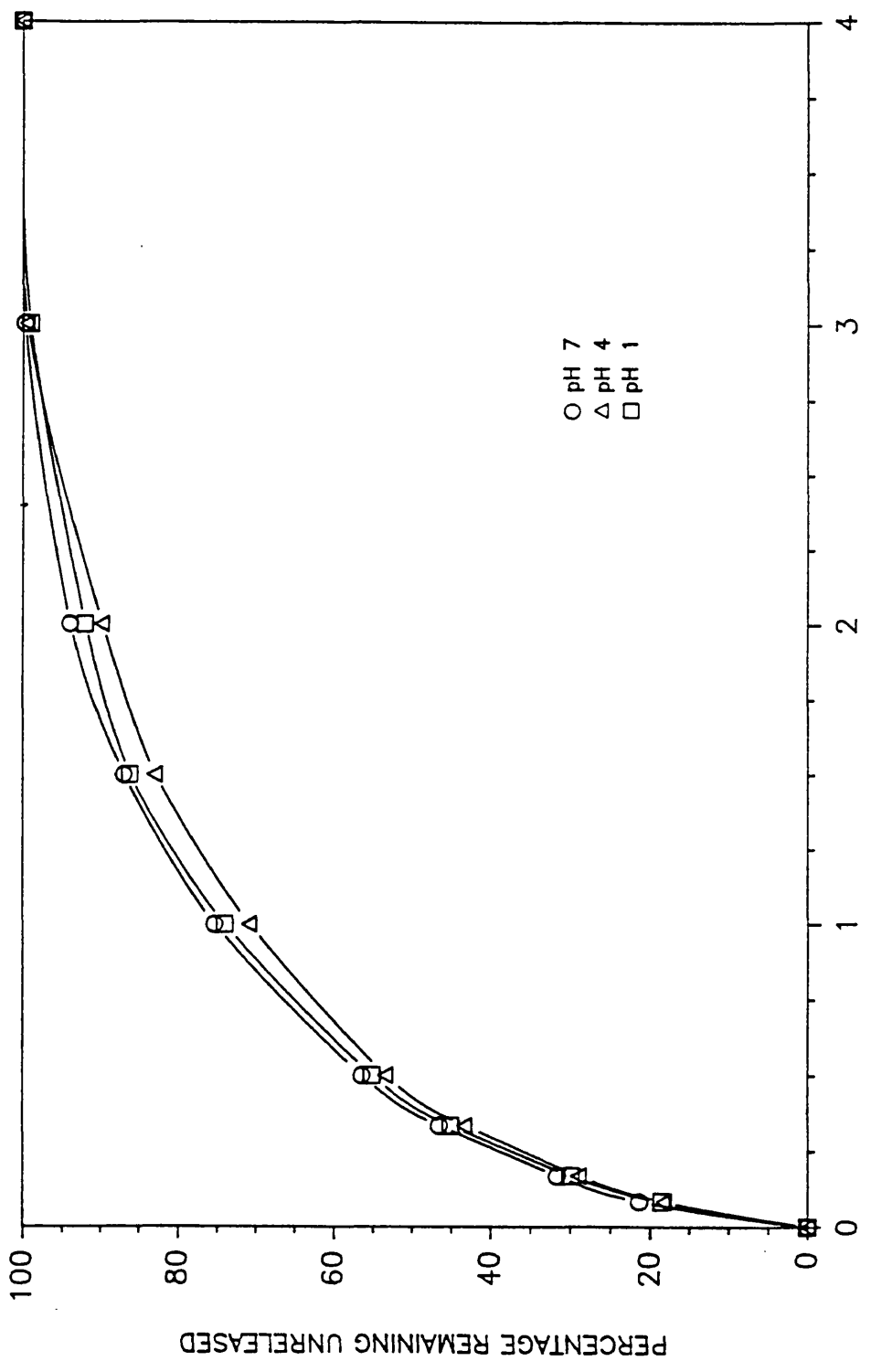


FIGURE 5 : IN-VITRO RELEASE FROM UNCOATED 80.0% THEOPHYLLINE PELLETS AS A FUNCTION OF pH

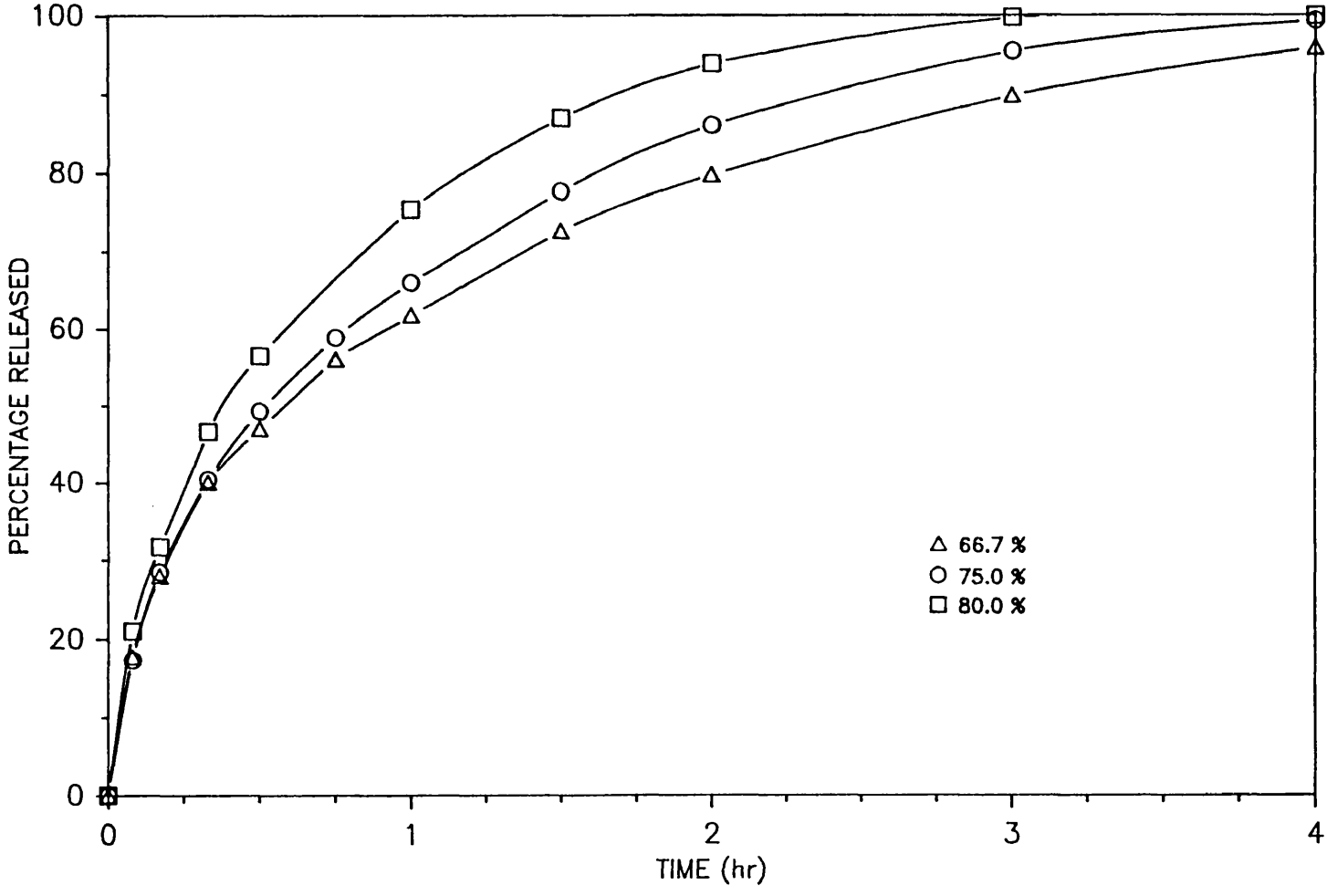


FIGURE 6 : IN–VITRO RELEASE FROM DIFFERENT UNCOATED THEOPHYLLINE PELLETS (pH 7)

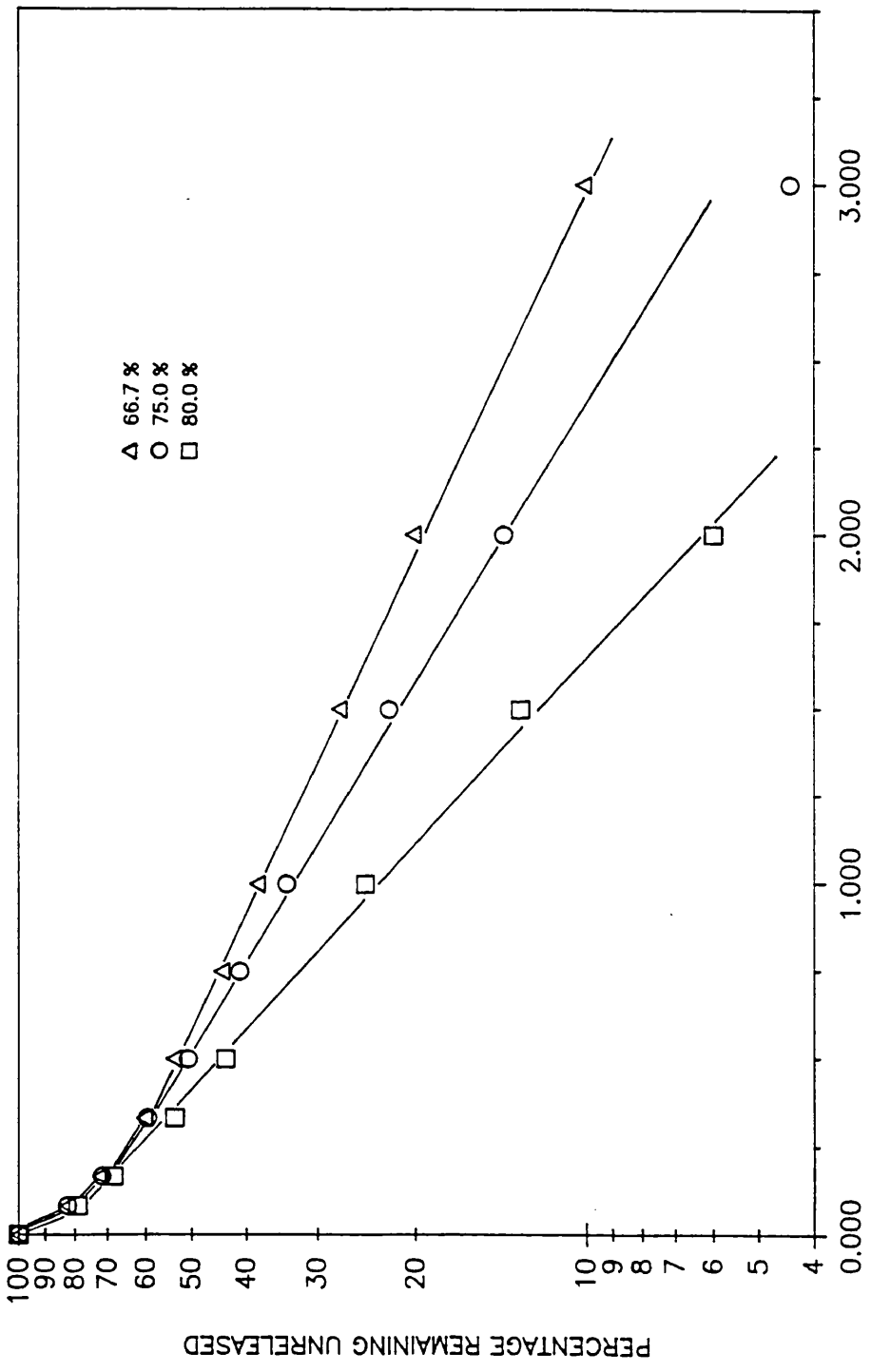


FIGURE 7 ; LOG PERCENTAGE REMAINING UNRELEASED VERSUS TIME PLOTS OF DIFFERENT UNCOATED THEOPHYLLINE PELLETS (pH 7)

The formulae of the pellets as used in the present study are indeed simple. Except for lactose in the 80% theophylline pellets, the only other excipient used is Avicel. However, Hermen *et al* (1989) recently reported that a combination of theophylline and Avicel can lead to instability of drug release on storage. A reason for the instability is attributed to the conversion of anhydrous theophylline to its more slowly soluble monohydrated polymorph during aqueous granulation or storage in humid conditions (90% relative humidity). In addition, it was postulated that the presence of moisture may initiate the formation of additional bonds between theophylline and Avicel, resulting in a reduction in the drug release rate. They showed that the moisture could either be derived from the humid storage environment or created by the crystal transition of theophylline from the monohydrated to the anhydrous form when stored in a low humidity condition. In the present study, evaluation of the 80% theophylline pellets was repeated after storage for 6 and 12 months in a screw capped bottle at normal room conditions (10 - 25 °C). The results shown in Figure 8 indicate that the drug release is stable. These contrasting observations may be due to the different drying conditions employed. Whilst Hermen *et al* (1989) used oven drying at 40 °C for 3 hours, the pellets of the present study were dried using the more efficient fluidized bed drier at 60 °C for an hour. Therefore, it can be perceived that with the drying and storage conditions employed in the present study, stability of drug release can be assured. Stability of release was also achieved by these workers when their products were stored under low humidity.

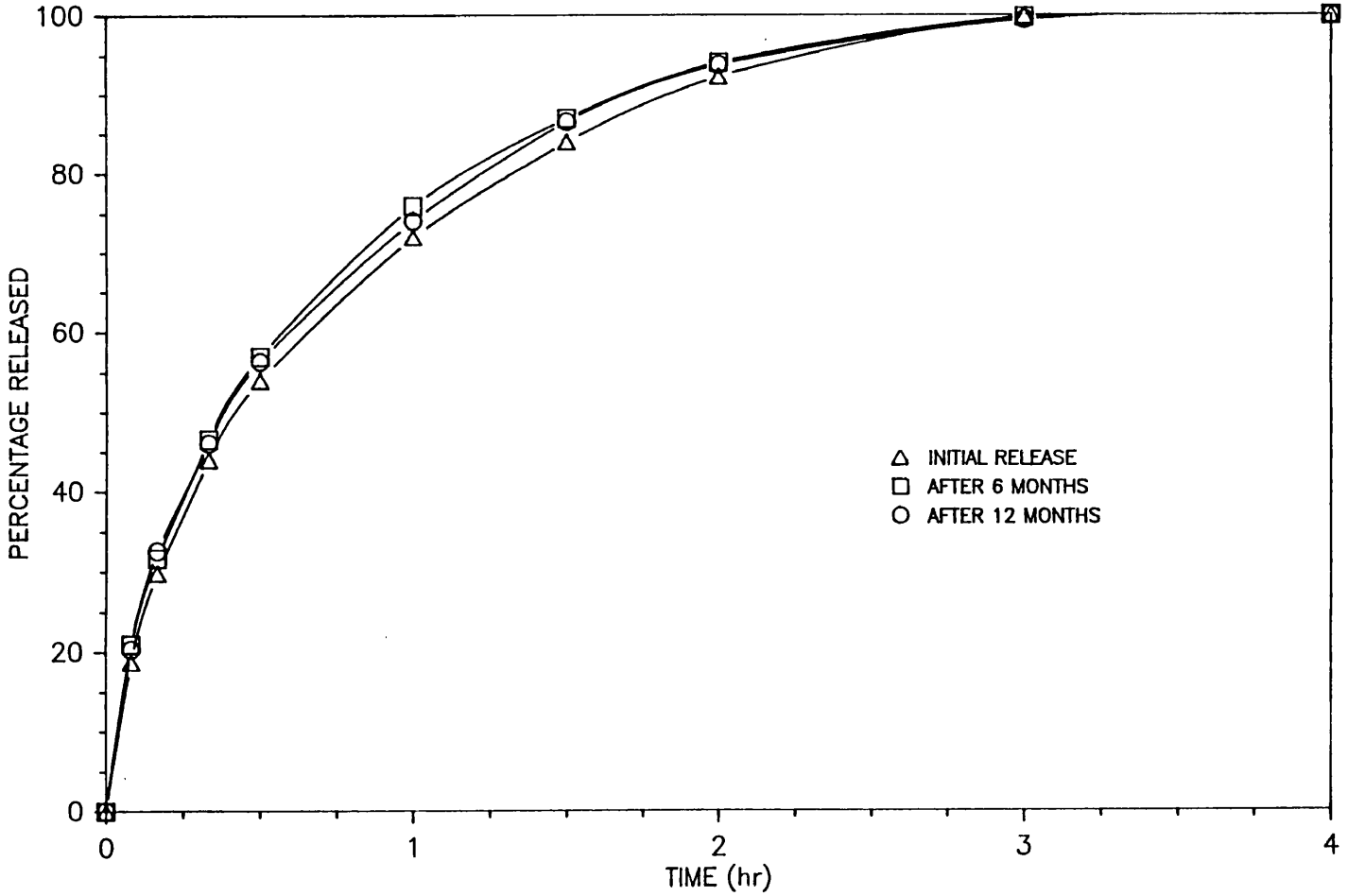


FIGURE 8 : IN-VITRO THEOPHYLLINE RELEASE FROM UNCOATED PELLETS AS A FUNCTION OF STORAGE TIMES (THEOPHYLLINE CONTENT OF PELLETS = 80.0 %)

2.4 CONCLUSION

In conclusion, theophylline pellets of high drug loading could be satisfactorily produced using the extrusion and spheronisation technique. A high yield was obtained in the size range of interest. The pellets are smooth and spherical, and have adequately rapid rate of dissolution making them suitable for use as a multiunit sustained release preparation after application of a rate-controlling polymer coat.

Chapter 3: DEVELOPMENT OF FILM COAT AND PREPARATION OF A NOVEL SUSTAINED RELEASE THEOPHYLLINE FORMULATION

3.1 INTRODUCTION

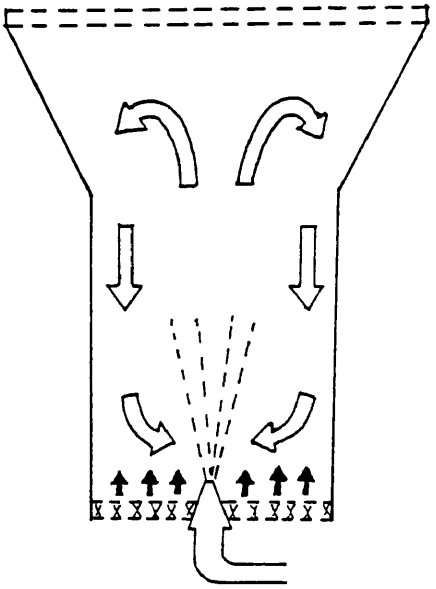
The theophylline pellets were film coated to provide a controlled release of the drug. Being spherical, the pellets possess the ideal shape for application of the coat in view of their low surface area to volume ratio. Indeed, this design is one of the most reliable forms of drug release, provided the coating polymer is totally insoluble regardless of the pH of the surrounding fluid (*Shangraw* 1988). Its drug release mechanism has been discussed previously in Section 1.1.1. The coat which acts as the rate-controlling barrier can be varied in thickness and/nature to achieve the desired porosity and permeability, and hence the rate of drug release. Ethylcellulose is a water insoluble polymer which is most widely used for this purpose. It is non-toxic, tasteless and odourless, and has good film forming properties, producing tough flexible films which are stable to both light and heat (*Rowe* 1985). Although used alone in some formulations, it is also used in combination with water-soluble polymers to alter the porosity of the coat.

A fairly recent innovation in pharmaceutical coating technology is the development of aqueous colloidal dispersions of the water insoluble coating polymers. The availability of these aqueous systems or pseudolatexes, enables the polymers to be cast without the use of organic solvents, thus avoiding the associated problems of toxicity, flammability and environmental pollution (*Ozturk et al* 1990). Indeed such aqueous polymeric dispersions have steadily replaced organic-based systems in all aspects of coating technology (*Yang and Ghebre-Sellassie* 1990).

In the present study coating was performed using a fluidized bed coating technique. In this method, air is introduced from the bottom of the coating chamber, which flows upwards to fluidize the pellets. The coating dispersion is then sprayed onto the pellets in a steady stream of air from an atomizer, which is usually located centrally at the bottom of the chamber. Careful adjustments of the flow rate of the fluidization air and the atomizing air will produce a uniform flow pattern of the pellets. An upward movement of the fluidized pellets in the

centre of the bed is promoted by the atomizing air. Since the top part of the coating chamber is widened, air pressure is reduced in this area, and the pellets upon reaching the top will flow back down along the side to the bottom to be resprayed as shown in the diagram below. This movement of the pellets together with a proper feed rate of the coating dispersion will ensure that the pellets are uniformly coated.

Diagram showing movement of pellets in the coating chamber



The film formation process can be summarized by the following sequence of events (*Steuernagel* 1989). When the solid polymer particles from the spray are initially deposited on the substrate, they exhibit Brownian movement. Capillary forces resulting from surface tension between water and air push the particles to polymer-polymer point contact creating a closer packing of the particles with some water still filling the voids. Interfacial tension between polymer and water causes further coalescence of the particles into a continuous film as all the water is evaporated and the particles deformed. Upon ageing of the film, the chain ends of the polymer molecules inter-diffuse across what used to be the boundary between the solid polymer particles and knit the polymer into a homogenous and continuous film.

It is apparent that during the film formation process the solid polymer particles have to undergo deformation and coalescence. Plasticization aids in overcoming their resistance to deformation and coalescence, and significantly reduces the critical film formation temperature (*Steuernagel* 1989). It also increases the elasticity and toughness of the film formed, so that

it is able to withstand cracking due to stress created internally or externally (*Porter* 1982). Depending on the degree of coalescence of the polymeric particles achieved during the coating process, further curing of the coat may be required to obtain complete film formation. The temperature dependency of the time required for complete film formation is well established. In one study, the pseudolatex films were shown to require 4 weeks to undergo complete curing at room temperature. However, at 60°C, the curing time was reduced to 1 hour (FMC literature). The implication of this finding is obvious, particularly when the films are used for controlled release purposes. If the films are not adequately cured during the coating process, the drug release characteristics may change on ageing of the film during storage.

In this section, the preliminary experiments necessary to develop a suitable film coat are described. The development of the pellet coating entailed a number of stages. Ethylcellulose as the sole film former was first examined. This was followed by evaluation of several coating additives for improving the permeability of the coat. When a satisfactory film coat was achieved, it was used to prepare a sustained release formulation with the theophylline pellets. Further evaluations were then conducted to assess the reproducibility of the manufacturing process, stability of the coat and pH dependency of drug release. The sustained release formulation was prepared using pellets of 80.0% theophylline content because of their high drug loading and more rapid rate of dissolution (Figure 6). However, during the development of the film coat, evaluations were conducted using pellets of 75.0% theophylline content because the 80.0% pellet formula was still being developed.

3.2 MATERIALS AND METHODS

3.2.1 MATERIALS

Ethocel AQ (Dow Chemicals, USA)

methylcellulose viscosity grade ≈15 cp (Fluka Chemie A G, Switzerland)

methylcellulose viscosity grade ≈400 cp (BDH Chemical Ltd., England)

polyethylene glycol 400 (BDH Chemical Ltd., England)

polyethylene glycol 4000 (BDH Chemical Ltd., England)

acacia B.P. (MaCarthys Ltd., UK)

sodium chloride G.P.R. (BDH Chemical Ltd., England)

3.2.2 GENERAL METHOD OF COATING

A commercially available aqueous dispersion of ethylcellulose (Ethocel AQ, DOW chemicals, USA) was used in the coating of pellets. It contains approximately 18.7% of the polymer and 6.2% of plasticizer. Coating was performed using an Aeromatic AG Strea 1 fluidized bed coater (ACM Machinery, Tadley), and operated under the following optimized conditions in all coating processes.

Atomizing air pressure	0.4 bar
Feed rate of coating dispersion	≈3G/min
Fluidization air (fan capacity)	18 units
Inlet temperature	60°C
Drying temperature	60°C
Drying time	1 hour

The weight of pellets used was 50G in each case. A small amount was used each time because of the limited quantity produced and also the large number of studies to be performed. The above operating conditions were chosen based on preliminary studies and found optimal for the 50G load.

3.2.3 STUDY ON ETHYLCELLULOSE FILM

Ethylcellulose as the sole film former was evaluated by coating pellets of 75.0% theophylline content with the polymer and then assessing the drug release characteristics. A series of

products with different film thicknesses were prepared. This was accomplished by varying the amount of coating dispersion sprayed. The film thickness is expressed as the theoretical weight gain of the polymer, calculated from the amount of ethylcellulose used relative to the weight of the pellets.

Seven film thicknesses were examined, corresponding to theoretical weight gains (TWG) of 1.9, 2.5, 3.8, 5.0, 7.5, 10.0 and 12.5%. In each case the amount of polymer dispersion required was measured, and diluted in a ratio of 2:1 with deionized water before use. The mixture was stirred for 5 minutes and used immediately. Coating of the pellets was performed according to the procedure mentioned above.

3.2.4 STUDY ON COATING ADDITIVES

Six water soluble compounds were examined as potential coating additives for improving the permeability of the ethylcellulose film. They included polyethylene glycol 400 (PEG 400), polyethylene glycol 4000 (PEG 4000), methylcellulose of viscosity grade 15 cp (MC 15), methylcellulose of viscosity grade 400 cp (MC 400), acacia and sodium chloride. These were chosen because of their different solubility rates. The coating additive to ethylcellulose ratio used were 0.8 for PEG 400 and PEG 4000, 0.4 for acacia and sodium chloride, 0.32 for MC 15 and 0.16 for MC 400. These ratios represent the highest amount of the individual compounds that could be incorporated without adversely affecting the coating dispersion or process as determined from preliminary experiments. Sodium chloride has the tendency to cause precipitation of the coating polymer, while the others can cause an increase in viscosity and tackiness of the dispersion, leading to easy agglomeration of the pellets.

All these compounds were dissolved in an appropriate volume of deionized water prior to mixing with a measured volume of Ethocel AQ, to yield a 1:2 dilution of the polymer dispersion. The mixture was stirred for 5 minutes and used immediately for coating. The effects of coating additives were studied using two coat thicknesses, corresponding to 5.0% and 12.5% TWG of ethylcellulose. Both these thicknesses were shown to be highly impermeable in the previous study (Figure 9) and therefore lend themselves for better studying the effects of the coating additives. As with the study on ethylcellulose film, theophylline pellets of 75.0% drug content were used as the drug cores, and were coated using the procedure described. Dissolution studies were then performed to evaluate the drug release

characteristics of the preparations.

3.2.5 PREPARATION OF SUSTAINED RELEASE THEOPHYLLINE FORMULATION AND CURING OF FILM COAT

From the results of the two preceding studies, the ethylcellulose-methylcellulose(400) coating mixture was chosen to prepare the final sustained release formulation with pellets of 80.0% theophylline content. Several products of different coat thicknesses were prepared. The TWG of the coat (ethylcellulose plus MC 400) were 2.3%, 2.9%, 3.5%, 4.1%, 4.7% and 5.8%. After coating, the products were stored in a screw capped container at normal room conditions (10-25°C). However, half the amount of the products with film thicknesses of 2.3%, 2.9%, 4.1% and 4.7% was subjected to further curing at 60.0°C for 24 hours in an oven before storage in the same conditions. Drug release studies were performed on all the products shortly after preparation and repeated 6 months later. For the fractions that were subjected to additional thermal treatment, studies were also repeated after 12 months.

3.2.6 *IN-VITRO* DRUG RELEASE STUDIES

Drug release studies were performed using the method described in section 2.2.3, with 900 ml distilled water as the dissolution medium. In addition, two of the products with mixed ethylcellulose-methylcellulose(400) coat of film thickness 2.3% and 4.1% described in 3.2.5 were also evaluated at pH 1, pH 4 and pH 7 to determine the pH dependency of drug release.

3.3 RESULTS AND DISCUSSION

The *in-vitro* theophylline release profiles of ethylcellulose coated pellets are as shown in Figures 9 and 10. A clear association between rate of drug release and thickness of coat is discernable from the plots. This is consistent with the results of Donbrow and Friedman (1975) and Benedikt *et al* (1988). The rate of theophylline release is related inversely to the thickness of the coat, suggesting that the film is controlling the release process and the drug concentration gradient in the coating is linear. At a theoretical weight gain of 5.0% or more, the ethylcellulose film appears to be poorly permeable to the drug. In all cases less than 5% of the dose was released in 12 hours. Although the release profiles were essentially linear, they were excessively slow for effective oral sustained drug delivery. Other studies have shown that the *in-vivo* availability is poor and incomplete if the total drug release time is in excess of 12 hours. (*Benedikt et al* 1988).

In Figure 10 it is observed that when the amount of coating polymer was reduced to less than 5%, the rate of drug release was increased substantially. Almost complete dissolution was achieved at the film thickness of 1.9% after 12 hours. Whilst the rate of drug release could be increased by reducing the coat thickness, this was found to produce an unsatisfactory release profile. Both plots for the 1.9% and 2.5% film thicknesses show a fast release rate initially, but this diminishes rapidly after about two hours. A closer examination of the plot for 1.9% film thickness reveals that more than 70% of the dose was released in the first 3 hours. However, only about 20% was released over the next 9 hours, and even after 12 hours dissolution was incomplete. Clearly, a more linear release profile is required to attain a relatively sustained and constant blood level of the drug. Ideally, the rate should be zero-order or constant (*Rogers and Kwan* 1981).

A further drawback of the ethylcellulose film as observed from the studies, was the increase in sensitivity of the rate of drug release to changes in film thickness when the amount of coating polymer used was low. A small reduction in the amount of coating polymer was observed to cause a large increase in the release rate. From a practical standpoint, this is undesirable because the coating process variables would be more difficult to control. Any slight variation in film thickness between batches of the same product during production would be expected to lead to poor reproducibility of results.

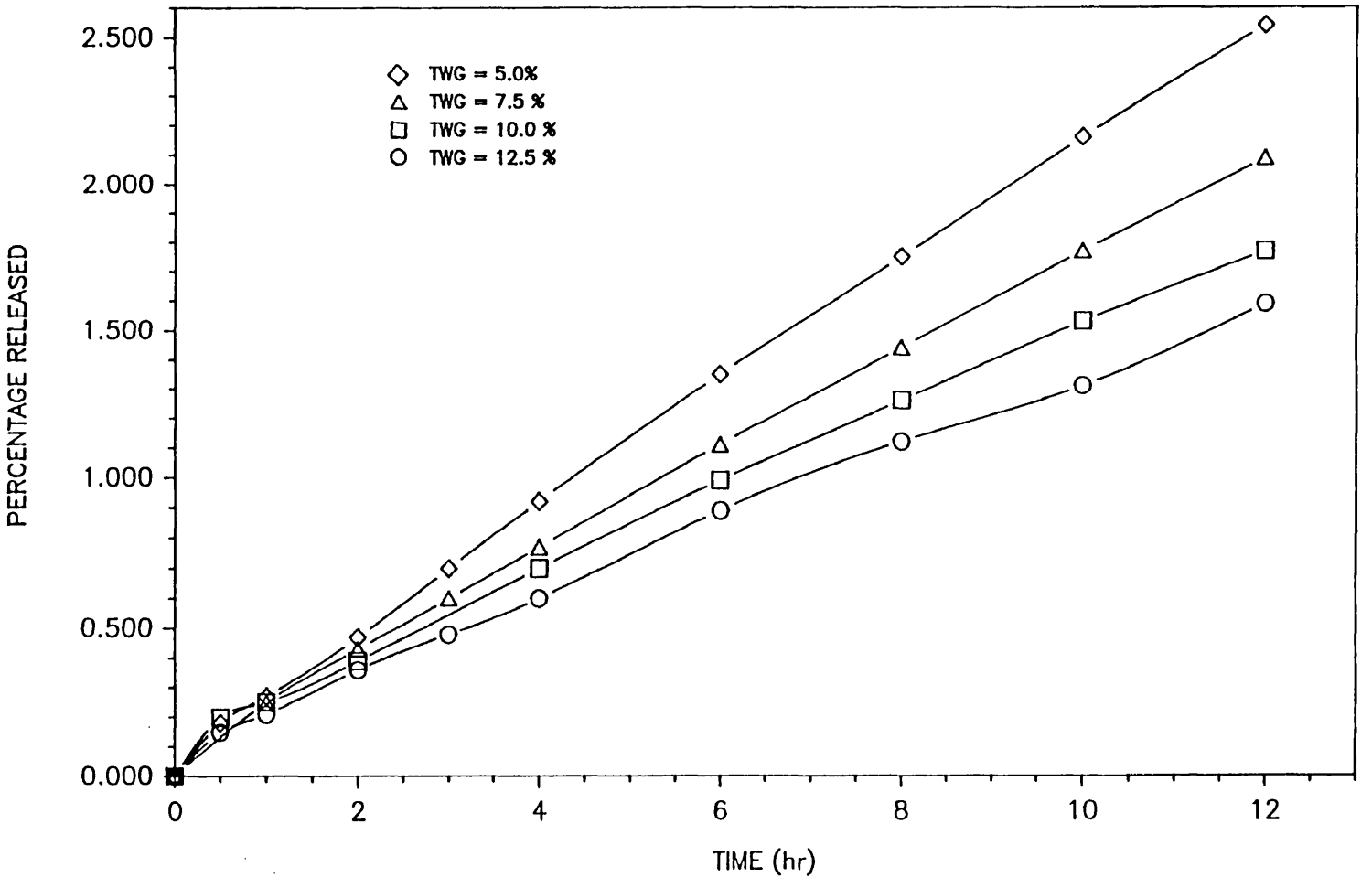


FIGURE 9 : IN–VITRO THEOPHYLLINE RELEASE FROM ETHYLCELLULOSE COATED PELLETS AS A FUNCTION OF COAT THICKNESS EXPRESSED AS THEORETICAL WEIGHT GAIN (TWG)

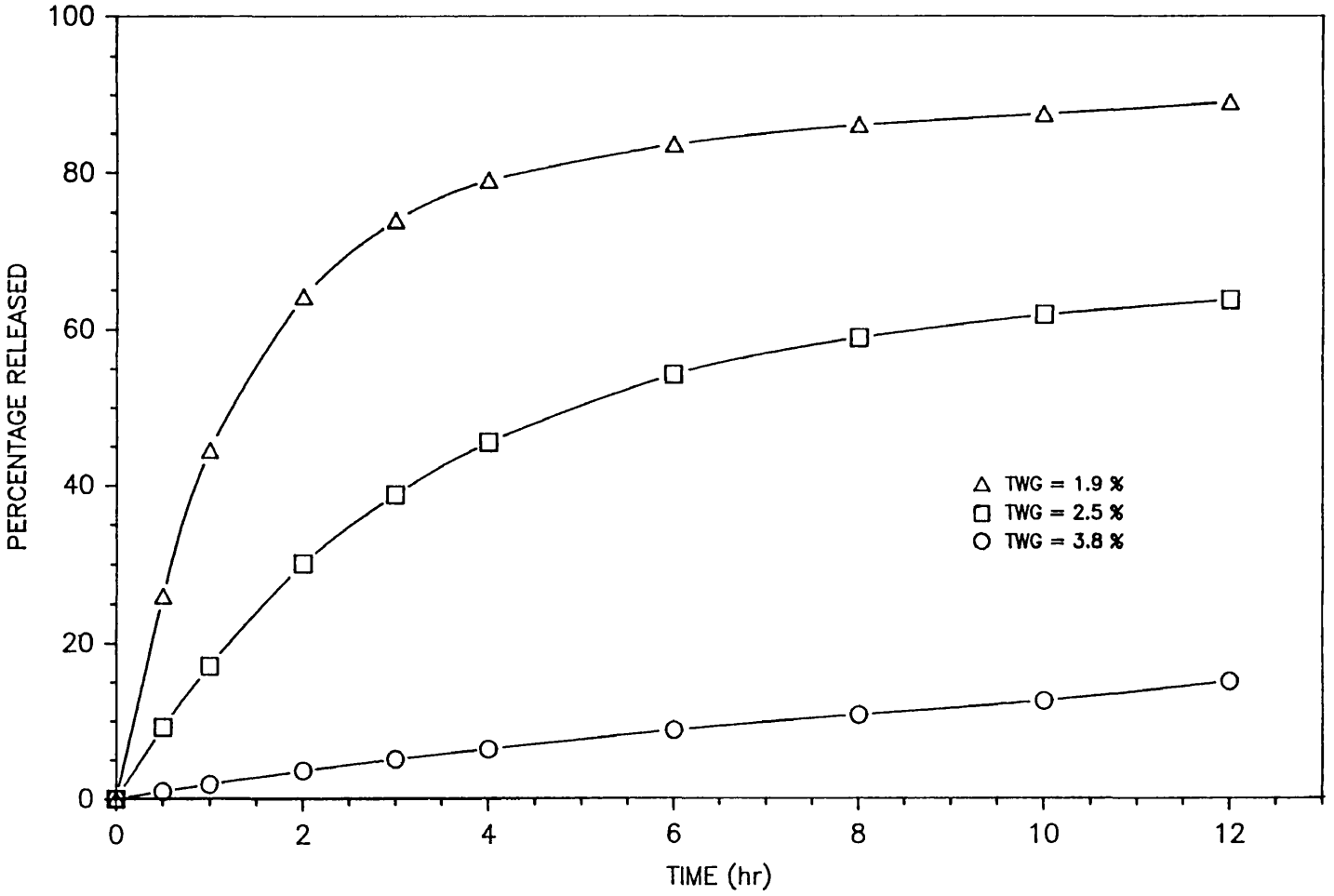


FIGURE 10: IN-VITRO THEOPHYLLINE RELEASE FROM ETHYLCELLULOSE COATED PELLETS AS A FUNCTION OF COAT THICKNESS EXPRESSED AS THEORETICAL WEIGHT GAIN (TWG)

On the basis of all these findings, it was concluded that ethylcellulose was unsatisfactory when used alone for the film coat. Therefore it was necessary to incorporate a coating additive to improve the drug release characteristics. It is interesting to note that in a recent study, Li *et al* (1990) also found ethylcellulose to be unsatisfactory as the sole coating material for preparing sustained release granules of theophylline. They also investigated the effects of several coating additives on the drug release from the ethylcellulose film. The additives studied included propylene glycol, carrageenin, methylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose (HPMC) and theophylline powder. Of these, HPMC was found to be most effective.

The six coating additives examined in the present study have very different solubility rates. Therefore it was interesting to examine how these differences could influence the permeability of the ethylcellulose film. The one that produced the most satisfactory drug release profile and ease of use in the coating process would be chosen to prepare the final formulation. Figures 11 and 12 show the theophylline release profiles of the pellets after incorporation of the additives. The film thickness of the preparations corresponds to 12.5% and 5.0% of ethylcellulose respectively for the two figures. Although the additives appeared to be effective in increasing the rate of drug release, the rates of increase varied. A comparison of Figures 11 and 12 also shows that the release rate was inversely related to the film thickness.

Of the additives used, PEG 4000 and MC 400 appear to be most effective and satisfactory in enhancing the film permeability. A linear release pattern was obtained with PEG 4000 (Figure 11). Although a deviation from linearity was observed when the coat thickness was decreased (Figure 12), there was a significant improvement in the overall release profile compared to the pure ethylcellulose film. The initial part of the plot in Figure 12 is linear for up to about 40% of the dose released and the subsequent decline in release rate is more gradual. It is interesting to note that when MC 400 was used, the release profile was different from that of the other coating additives. Referring to Figure 11 it can be seen that its release profile curves slightly upwards rather than downwards, with the release rate increasing with respect to time. This phenomenon is again observed in the initial part of the plot for a thinner film in Figure 12, with the overall profile remaining linear. It may also be noted that this feature is characteristic only with MC 400, but not with MC 15, and nor was this observation

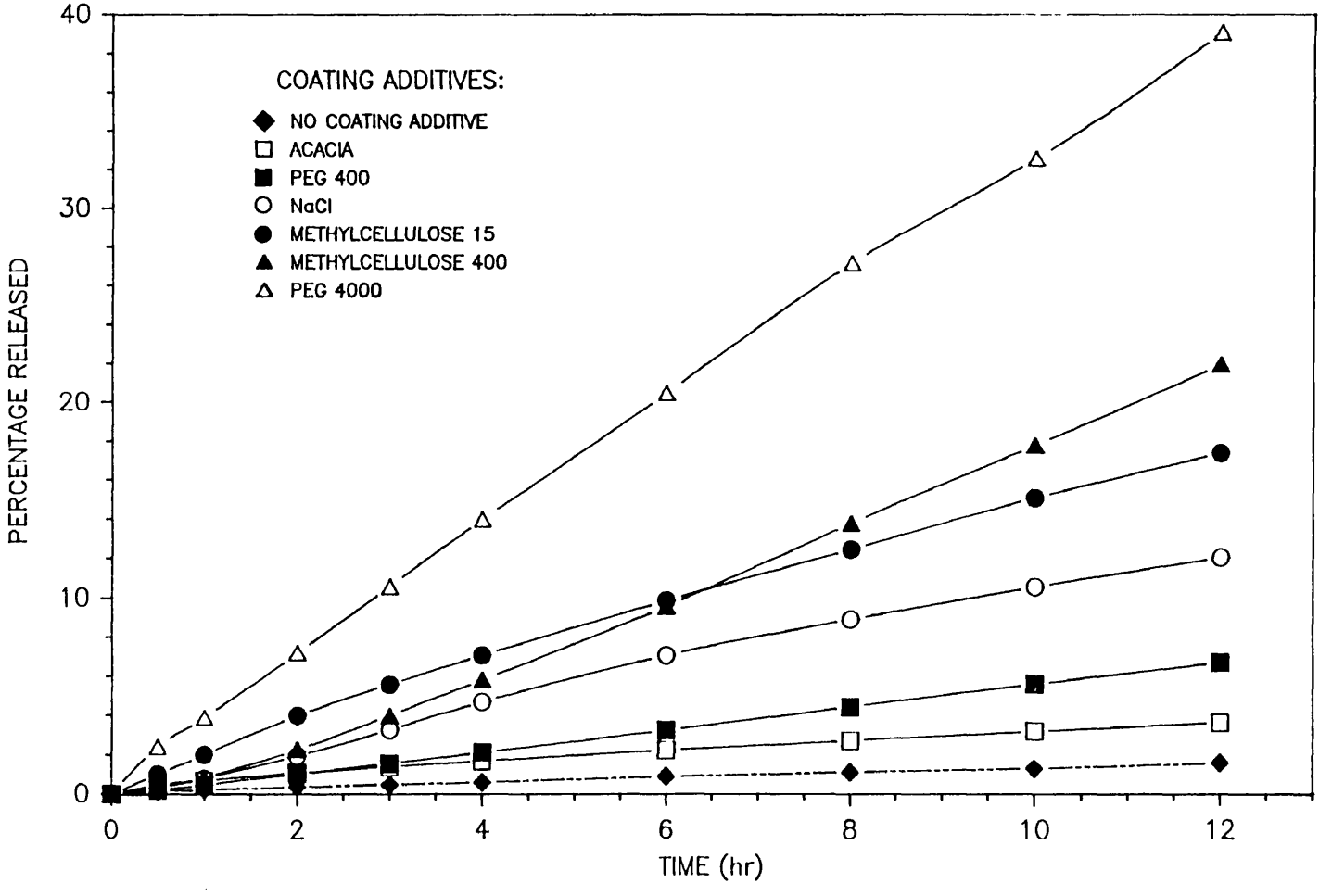


FIGURE 11: IN-VITRO THEOPHYLLINE RELEASE FROM ETHYLCELLULOSE COATED PELLETS WITH DIFFERENT COATING ADDITIVES (TWG OF ETHYLCELLULOSE = 12.5%)

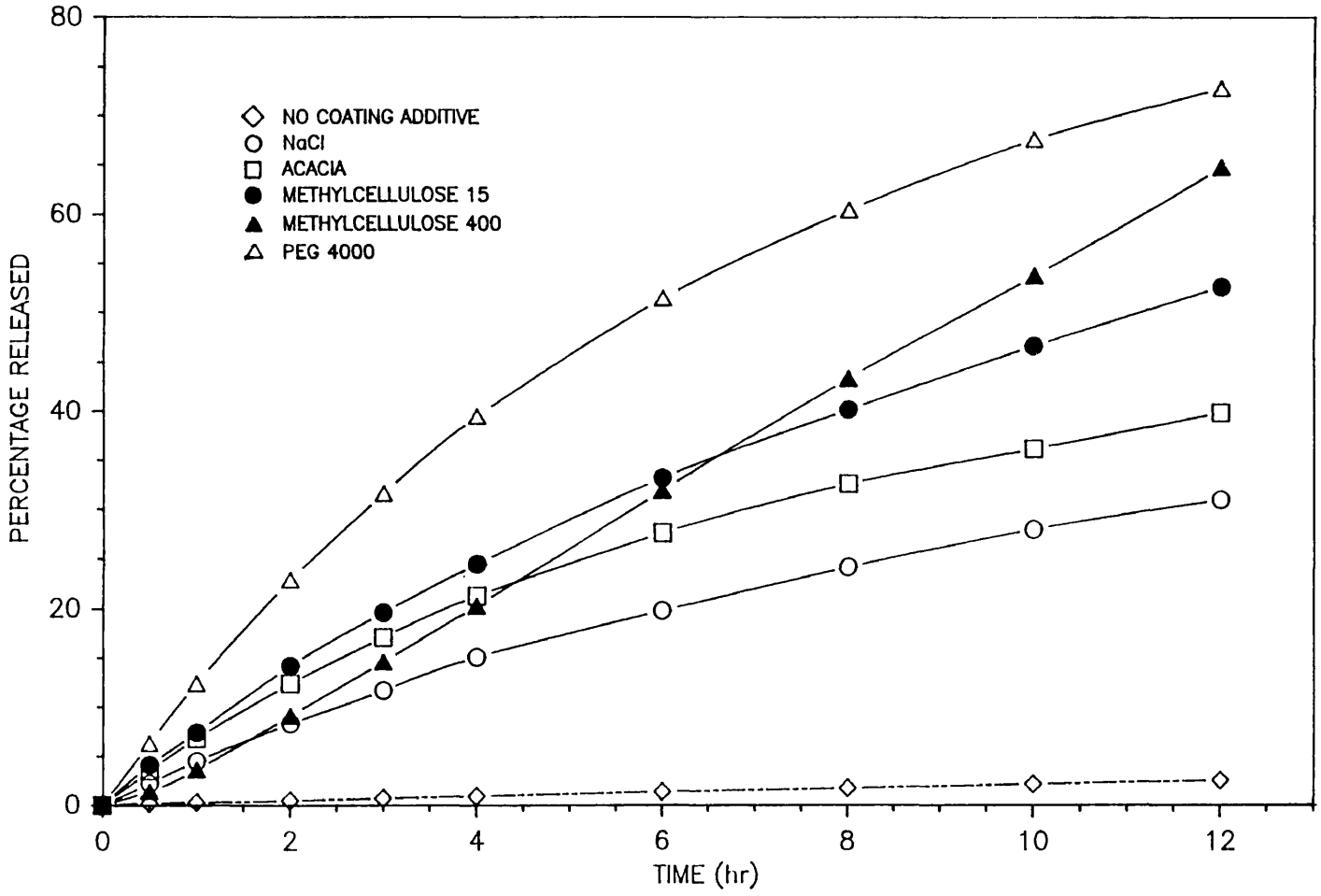


FIGURE 12: IN-VITRO THEOPHYLLINE RELEASE FROM ETHYLCELLULOSE COATED PELLETS WITH DIFFERENT COATING ADDITIVES (TWG OF ETHYLCELLULOSE = 5.0%)

made for the low viscosity grades methylcellulose studied by Li *et al* (1990) and Coletta and Rubin (1964).

As promising release profiles were obtained with both PEG 4000 and MC 400 a further comparison was made between the two and the results are as shown in Figure 13. The film thickness was carefully adjusted so that almost equal rates of drug release were obtained for the two compounds. It may be seen in Figure 13 that MC 400 has a more uniform release at both sets of release profiles compared. Therefore, MC 400 was chosen for use in the final sustained release theophylline formulation. Although PEG 4000 has been shown to be useful by Donbrow and Friedman (1974, 1975) and Donbrow and Samuelov (1980), their evaluations were mainly conducted using isolated films prepared by casting the polymer mixture onto a substrate such as glass or mercury. As a result, the films formed might be structurally quite different from those sprayed onto pellets or tablets and this could lead to differences in behaviour under test conditions (*Porter* 1982). Moreover, their methodology would not immediately reveal the problems that could arise during the coating process used here. In the present study, the addition of PEG 4000 into the polymer dispersion yielded a tacky mixture which caused agglomeration of the pellets during coating. This may in part, be due to the low melting point of PEG 4000 (53°-56°C) which is less than the coating bed temperature used. Lowering of the bed temperature would be undesirable as this might prolong the film formation time. However, no such problem was encountered with MC 400, and it was easy to use. At the low concentration employed, the slight increase in viscosity of the coating mixture did not interfere with the coating process.

With regard to the other compounds studied, MC 15 produced an increase in film permeability that was comparable to MC 400. However its release profile was not linear at either of the film thicknesses examined. Nonlinear release profiles were also observed with acacia, sodium chloride and PEG 400, and their ability to increase the permeability of the ethylcellulose film was considerably less than PEG 4000 or MC 400. A further disadvantage of PEG 400 was its tendency to cause agglomeration of the pellets, whilst sodium chloride on the other hand, tended to induce precipitation of the polymer dispersion, especially when a higher concentration was added. The latter may be more useful if the coating is applied using an organic based system as practised by Benedikt *et al* (1988) with the additive lactose.

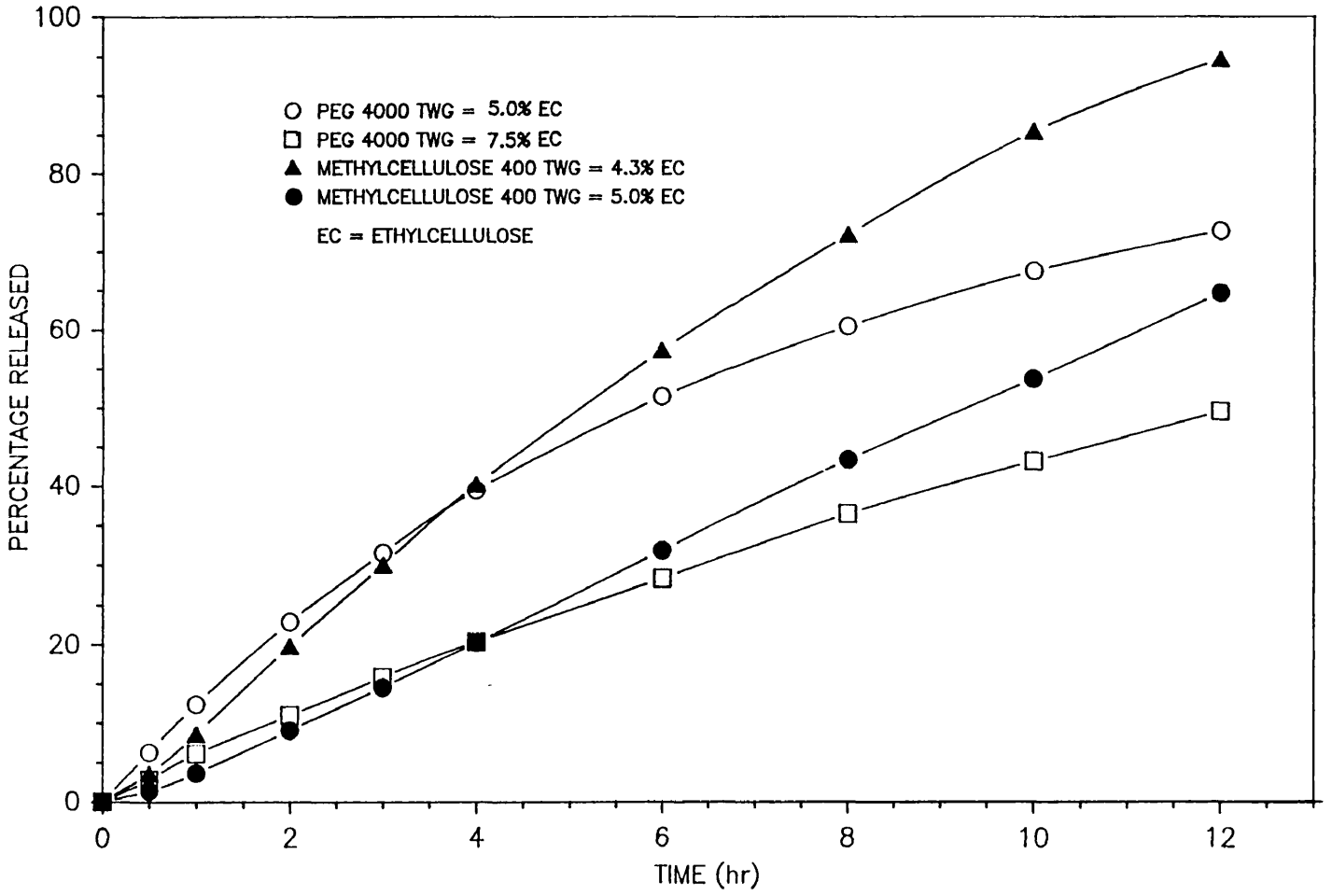


FIGURE 13 : IN–VITRO THEOPHYLLINE RELEASE FROM ETHYLCELLULOSE COATED PELLETS COMPARING THE COATING ADDITIVES PEG 4000 AND METHYLCELLULOSE 400

Donbrow and Friedman (1974) suggested that the increase in film permeability by the addition of a water soluble additive, is due to an enhanced hydrophilicity of the membrane when the additive is bound within the film. On the other hand, if the additive is dissolved out by the dissolution medium, the mechanism is one of increased porosity. In studies using mixed ethylcellulose-polyethylene glycol film, Lippold and Forster (1981) suggested that the dissolution of the soluble component created aqueous filled pores in the film, and that theophylline (which is a big molecule) permeated the film by diffusing through these pores. However, the drug release through the aqueous pores may be accompanied by the diffusion of the drug molecules through the continuous polymer phase (*Ozturk et al* 1990). Nevertheless, the contribution of this pathway can be considered negligible if the continuous polymer phase is highly impermeable.

Based on Fick's law the drug release from the coated pellets can be described by the following expression.

$$\text{release rate} = \frac{AD(C1-C2)}{L} \qquad (1)$$

where C1 is the dissolved drug concentration in the core, C2 the concentration in the surrounding medium, A the diffusional area, D the diffusivity of the drug in the medium, and L the thickness of the coat. According to the expression, if all the terms on the right of the equation are held constant, a zero order rate of release can be achieved. Thus, the linear plots obtained with some of the preparations is an indication that all these requirements had been met. In contrast, the deviation from linearity could be due to one or more factors not remaining constant as required, eg. when the concentration of drug in the core falls or when the dissolution of the core becomes slower than the permeation of the drug through the membrane. Under these conditions, a constant drug concentration (C1) can no longer be maintained, and the rate of drug release will start to decline. This may explain the nonlinearity of the later part of the plot for PEG 4000 in Figure 12, when more than 40% of the dose has been released. However the nonlinearity observed with sodium chloride, acacia, MC 15 and PEG 400 was unlikely to be due to a changing drug concentration in the core, since all the deviations occurred even at low percentages of drug released. For these cases, the deviation may be associated with a swelling of the film coat, resulting in an increase in

the diffusional path length L and a decrease in the effective area of diffusion A. On the other hand, the unique release profile observed with MC 400 in Figure 11 could be attributed to a slow and gradual leaching of the poorly soluble polymer from the film, such that the effective diffusional area and hence the film permeability was slowly increased with time. This may also explain the differences in the release profiles obtained with PEG 4000 and MC 400 shown in Figures 12 and 13. In the case of MC 400, the decline in drug concentration of the core was being compensated to a certain extent by the increasing film permeability, and hence the overall drug release rate remained fairly constant.

From the results obtained, MC 400 is a clear choice for preparing the final formulation with theophylline pellets of 80.0% drug content. A series of products with varying coat thicknesses were prepared and their drug release profiles are as shown in Figure 14. All the plots are essentially linear and it may also be observed that the release rate could be readily varied by altering the film thickness. For each of four preparations, two batches were prepared and their drug release profiles assessed. The release profiles of each batch are shown in Figure 15. It can be seen that there is satisfactory reproducibility between batches in all the four preparations. This signifies that the process variables could be adequately controlled and the production process was highly reproducible. The formulation was further evaluated under different pH conditions using two preparations of film thickness 2.3% and 4.1%. The results in Figure 16 show that the drug release is relatively unaffected by pH. This is in good agreement with the results of Benedikt *et al* (1988) obtained with ethylcellulose-lactose coated theophylline pellets,but differed from the findings of Lippold *et al* (1989), who reported changes in permeability of the polymer film at high pH (>6) due to the presence of a small amounts of carboxylic groups in the ethylcellulose used.

The drug release profiles as a function of storage time are shown in Figure 17 and 18 for the portions of pellets that were not subjected to additional curing of the film coat. It can be seen that the release rates of all the preparations were increased after 6 months. In contrast, the release profiles of the fractions that were cured for 24 hours remained stable even after 12 months of storage as seen in Figure 19. These results clearly indicate that additional thermal treatment is crucial for complete curing of the film coat so that the release will remain stable during storage. The increase in film permeability of the pellets that were not sufficiently

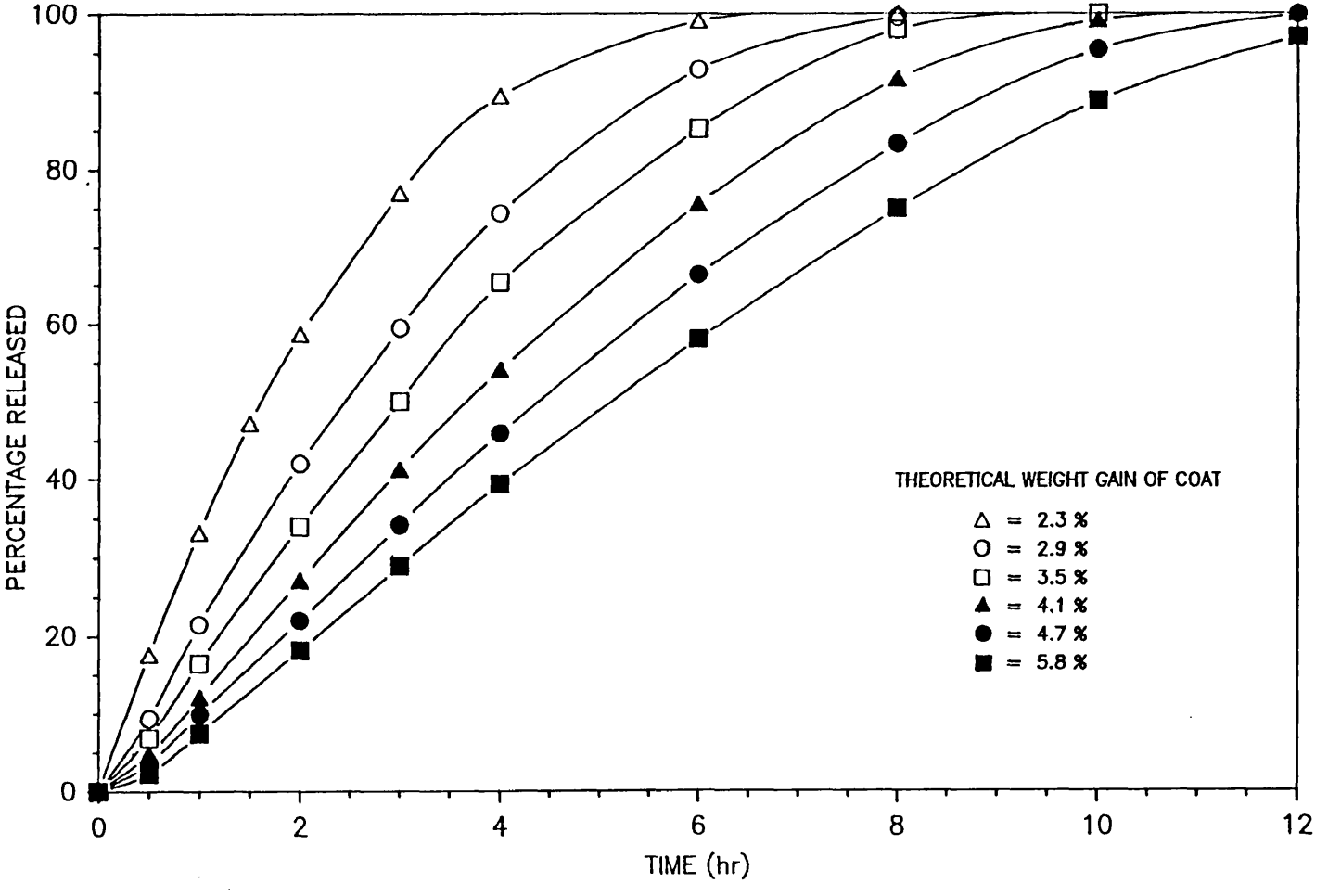


FIGURE 14: IN-VITRO THEOPHYLLINE RELEASE FROM ETHYLCELLULOSE-METHYLCELLULOSE (400) COATED PELLETS AS A FUNCTION OF COAT THICKNESS

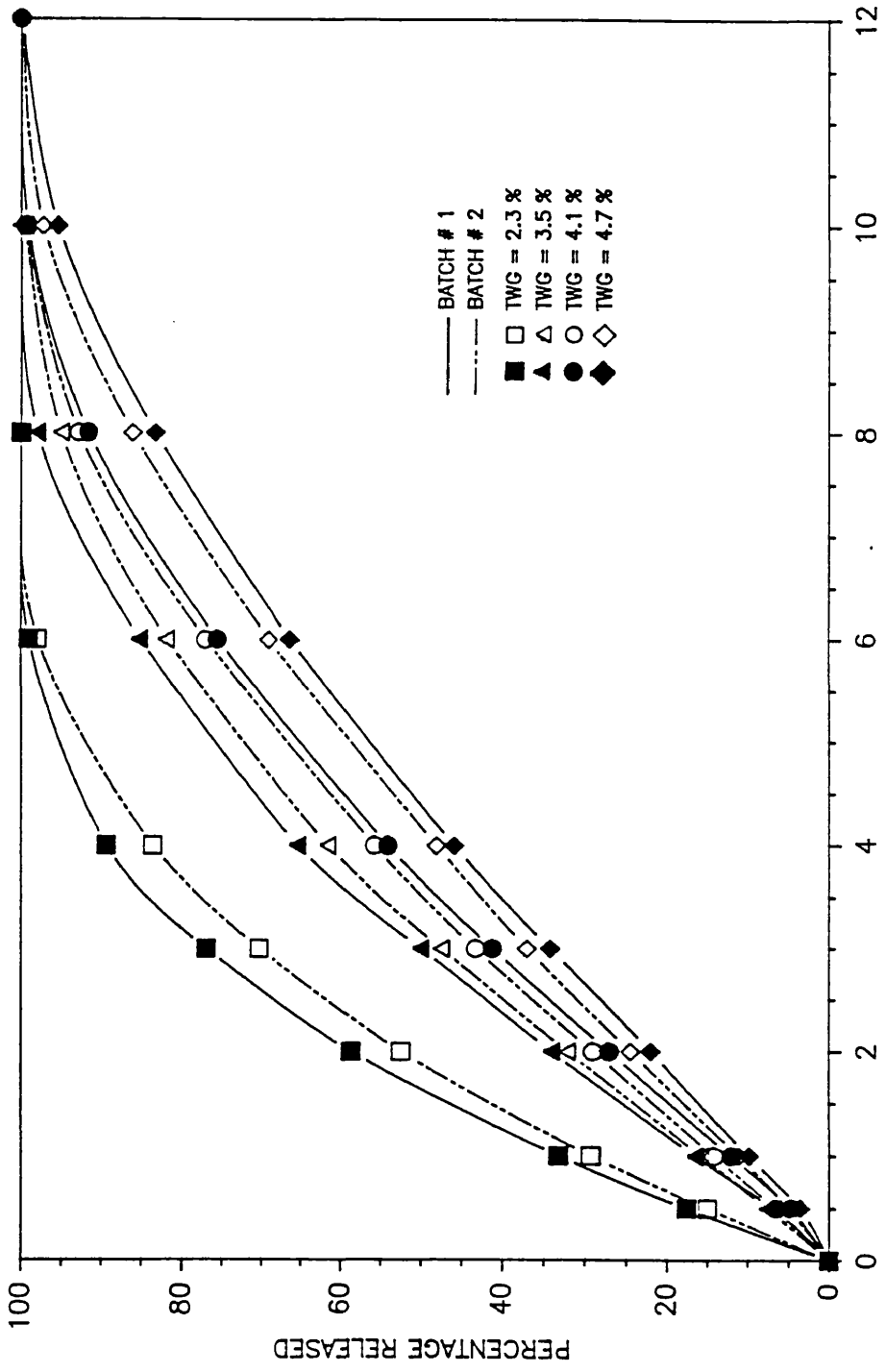


FIGURE 15: IN-VITRO THEOPHYLLINE RELEASE FROM DIFFERENT BATCHES OF COATED PELLETS

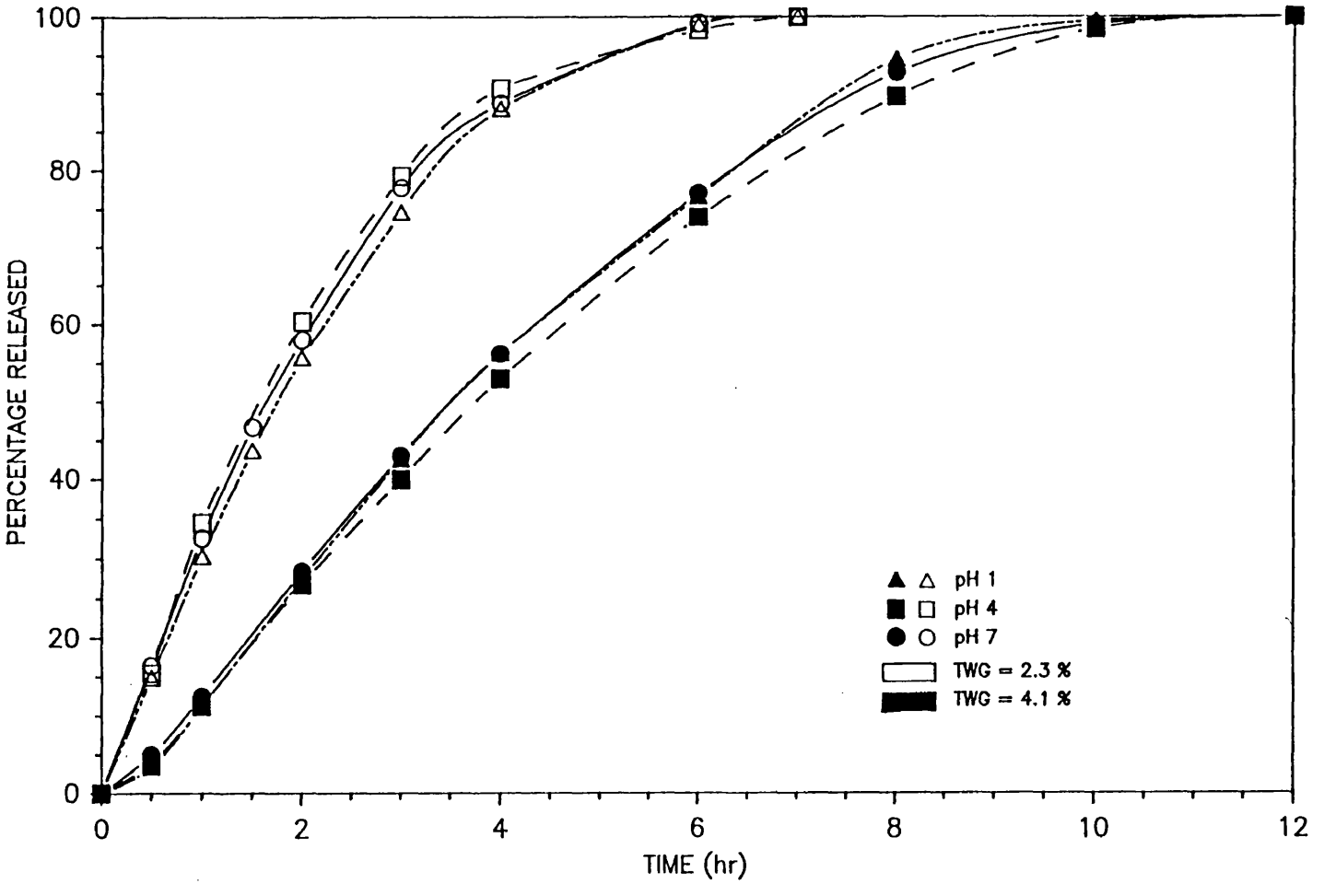


FIGURE 16: IN-VITRO THEOPHYLLINE RELEASE FROM ETHYLCELLULOSE-METHYLCELLULOSE(400) COATED PELLETS AS A FUNCTION OF pH

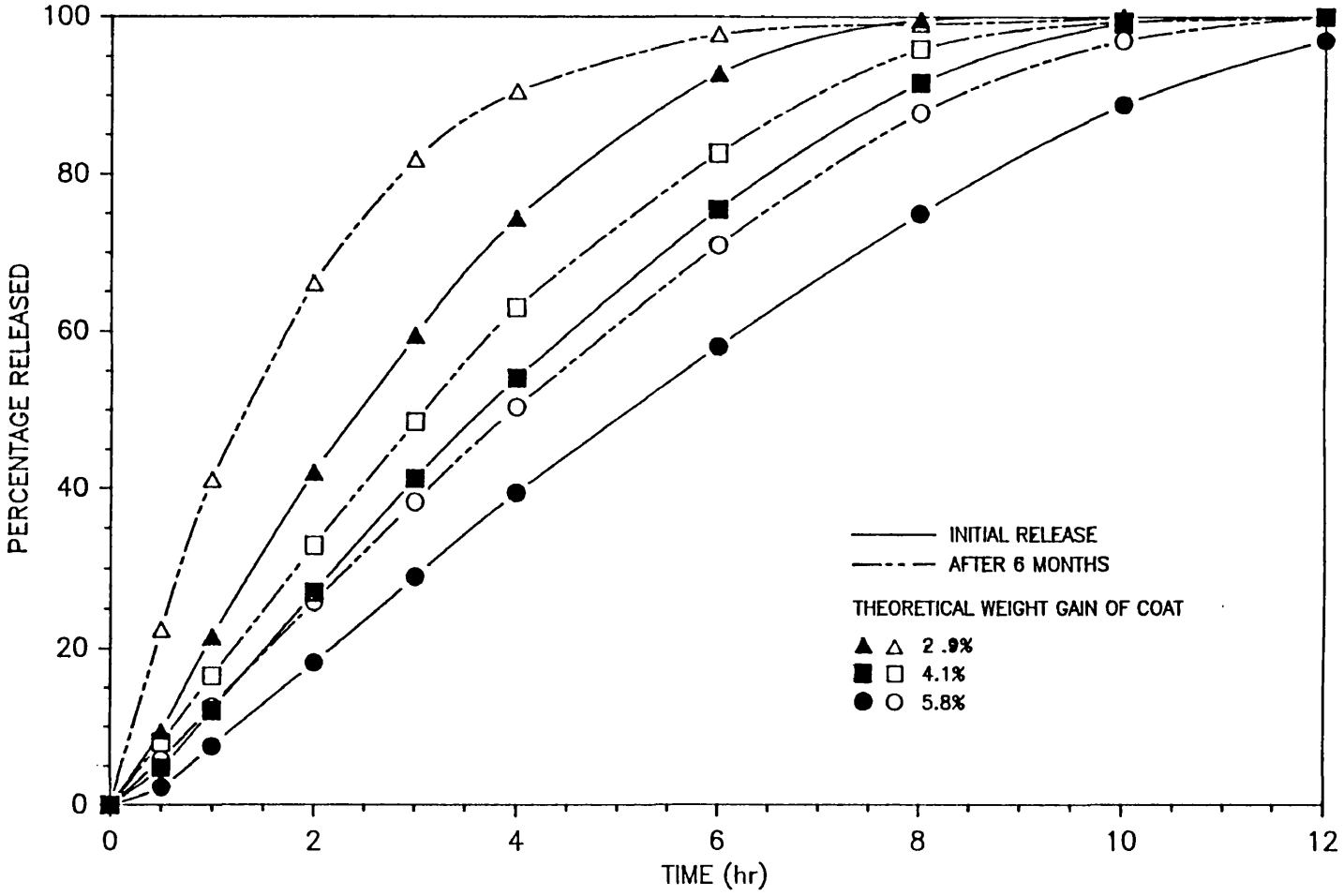


FIGURE 17: IN-VITRO THEOPHYLLINE RELEASE FROM COATED PELLETS WITH NO ADDITIONAL COAT CURING AS A FUNCTION OF TIME STORAGE TIME

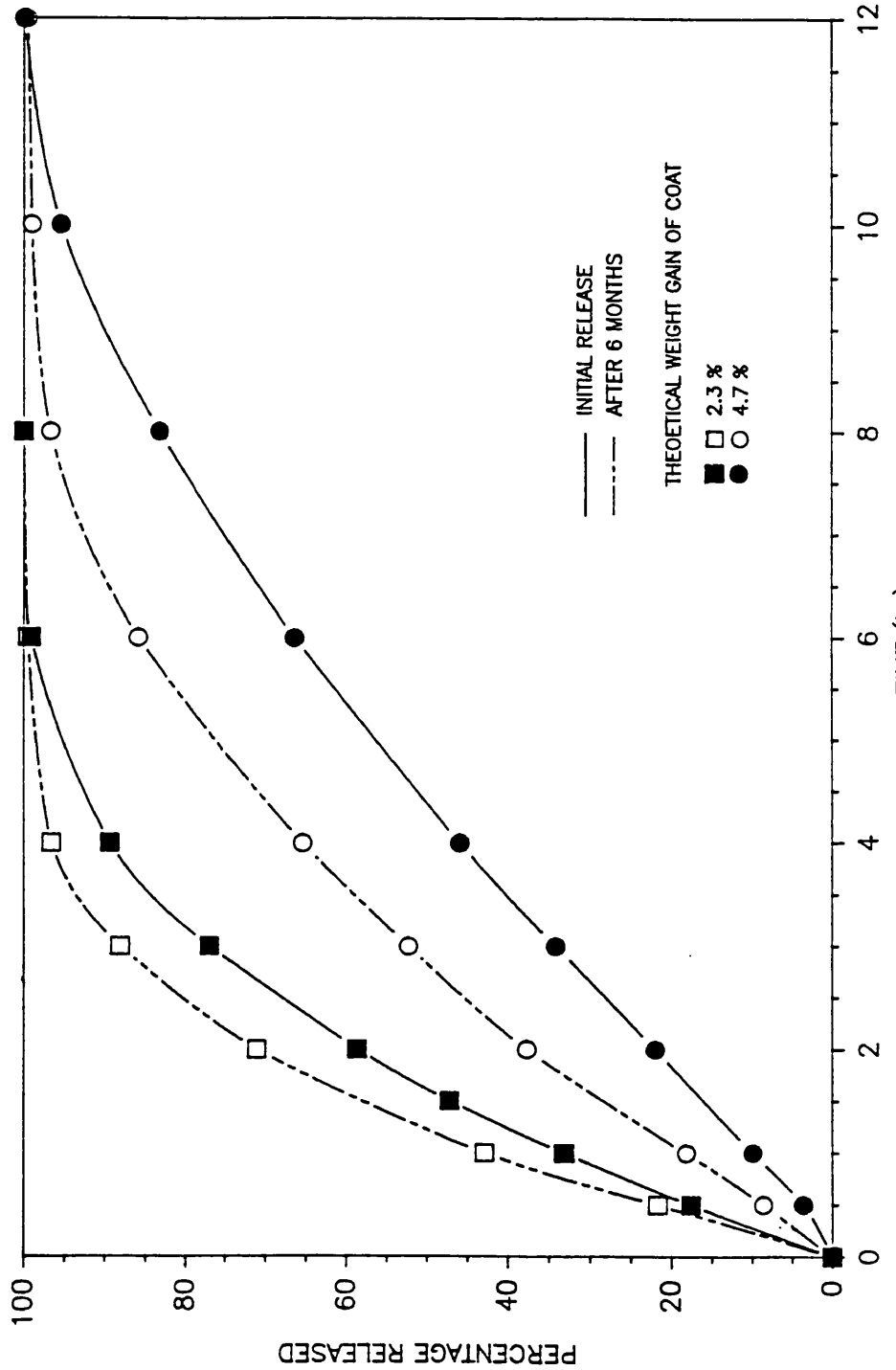


FIGURE 18: IN-VITRO THEOPHYLLINE RELEASE FROM COATED PELLETS WITH NO ADDITIONAL COAT CURING AS A FUNCTION OF STORAGE TIME

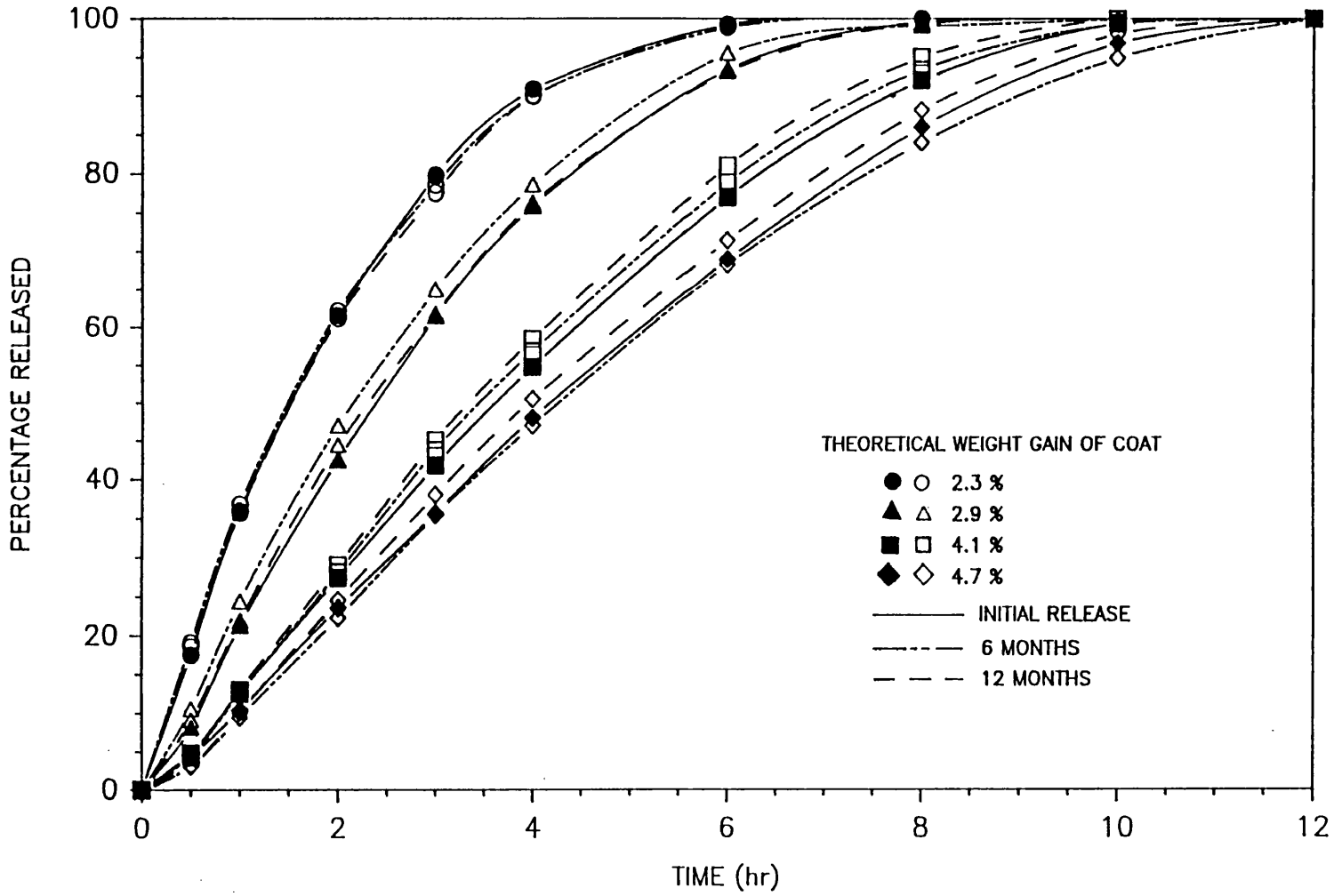


FIGURE 19 : IN–VITRO THEOPHYLLINE RELEASE FROM COATED PELLETS AFTER COAT CURING AS A FUNCTION OF STORAGE TIME

cured may be attributed to formation of micro-cracks or fractures in the coat during storage, possibly due to the presence of some weak linkages between the ethylcellulose particles because of incomplete interdiffusion after coating (*Lippold et al* 1989). The release profiles before and after curing are shown in Figure 20. The permeability of the coat was not affected by the additional thermal treatment. The results also show that an integral coat has been formed prior to the additional thermal treatment, except perhaps for a few weak linkages between the polymer particles that may yield some defects during storage.

Plate 1 shows the scanning electromicrographs of the pellet construction. The micrographs a and b were taken of the pellets before and after coating. It can be seen that the pellet surface is relatively rough before application of the coat (micrograph c). In contrast, the film deposited appears smooth and homogenous (micrograph d), indicating that the polymeric particles were adequately deformed and fused, to yield a continuous film under the coating conditions used. In addition, the film thickness also appears very uniform as seen with the 2.3% and 5.8% coatings in micrographs e and f respectively. A very thin film of approximately 5μ was obtained with the former, while the thickness of the latter was approximately 14μ.

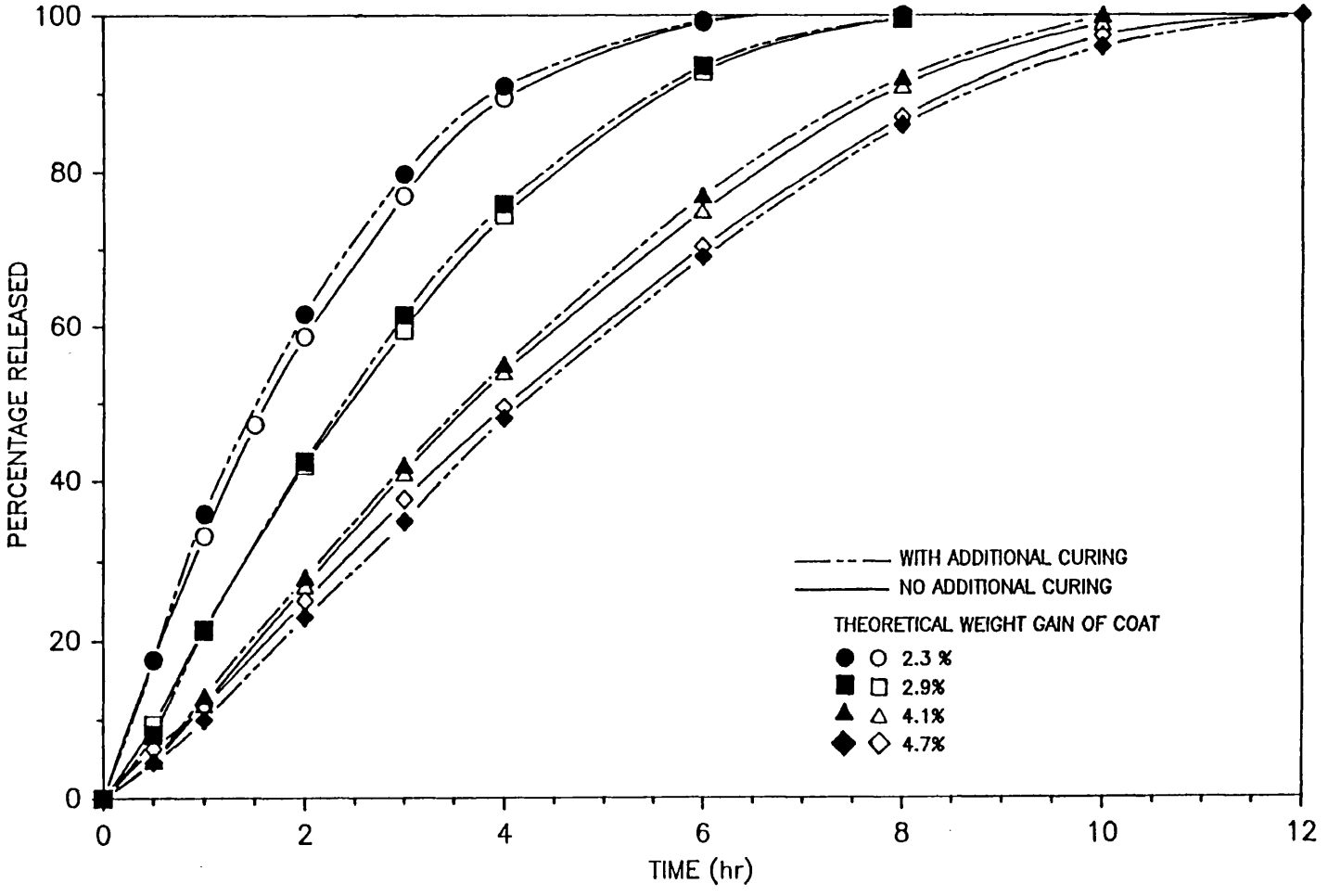
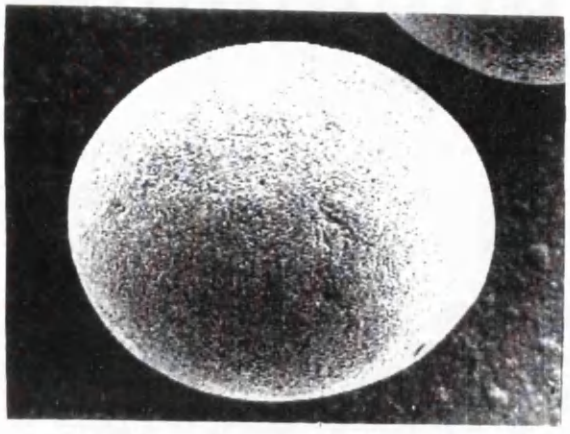
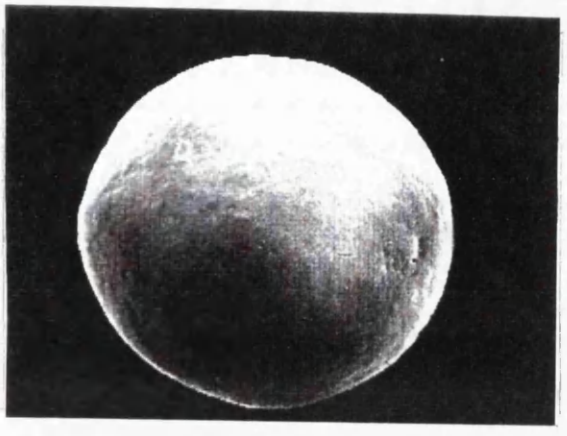


FIGURE 20 : IN-VITRO THEOPHYLLINE RELEASE FROM COATED PELLETS BEFORE AND AFTER COAT CURING

Plate 1 : SCANNING ELECTRONMICROGRAPH OF PELLETS



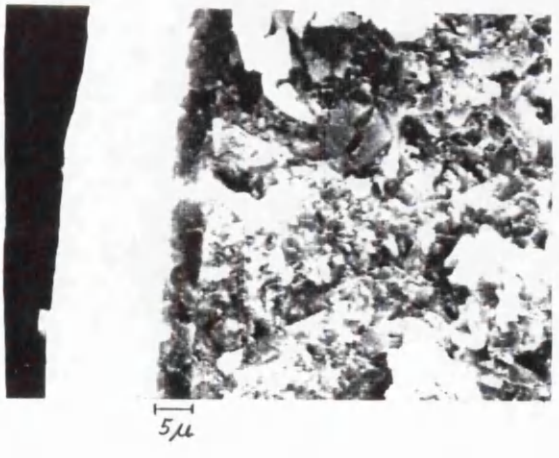
(a) uncoated pellet (X50)



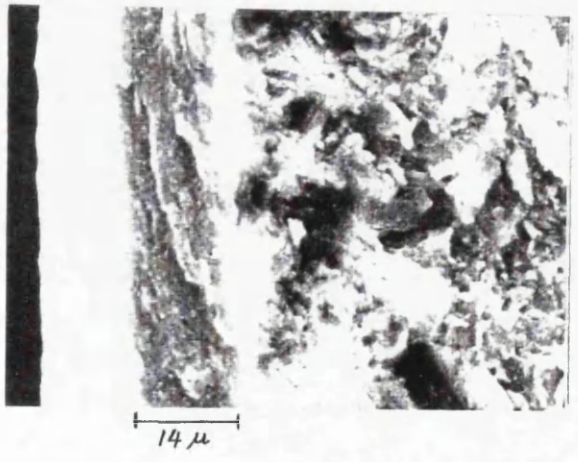
(b) coated pellet (X50)

(c) uncoated pellet surface (X500)

(d) coated pellet surface (x500)



(e) cross-section of coated pellet (X1000)
coat thickness = 2.3%



(f) cross-section of coated pellet (X1000)
coat thickness = 5.8%

3.4 CONCLUSION

In conclusion, a sustained release theophylline formulation with satisfactory *in-vitro* release characteristics was successfully prepared using the MC 400 coating mixture and 80.0% theophylline pellets. The rate of drug release is unaffected by pH and can be readily varied by manipulating the film thickness. Stability of release during storage can be achieved by additional thermal treatment of the coat. The manufacturing process shows good reproducibility as assessed by *in-vitro* drug release.

Chapter 4: *IN-VIVO* EVALUATION (STUDY I)

4.1 INTRODUCTION

The earlier *in-vitro* studies have provided an evaluation of the essential formulation variables during development of the dosage form. On the basis of these studies, a sustained release formulation of theophylline with satisfactory *in-vitro* dissolution characteristics was successfully developed. However, as mentioned previously, *in-vitro* tests per se cannot directly predict *in-vivo* performance, and validation of the sustained release claim is best achieved by *in-vivo* testing on human volunteers. A qualitative and quantitative comparison of the plasma/serum concentration profile with that of a reference preparation of the drug, such as a solution, will establish the sustained release characteristics of the dosage form: a standard procedure is to assess both the rate and extent of absorption. A variety of mathematical techniques have been used in this respect and are well reviewed by Cutler (1981). The results derived from such analyses, particularly those that can describe the *in-vivo* release or absorption versus time profiles, are both informative and useful for interrelating *in-vitro* and *in-vivo* data.

The aims of this part of the study therefore, were to evaluate the *in-vivo* performance of the formulation in healthy human volunteers and to determine if any association could be established between the *in-vitro* and *in-vivo* results. Results of previous investigations on multi-unit coated dosage forms by Benedikt *et al* (1988), showed that formulations which dissolved completely *in-vitro* in 6 hours or less, would behave more like conventional formulations *in-vivo*. Conversely, formulations with a total *in-vitro* dissolution time of 12 hours or more, would produce a sustained level of drug in plasma but would be incompletely absorbed *in-vivo*. On this basis, two products which had been formulated as described in section 3.2.5 were chosen for this study. The products differed solely in their coating thickness which was 4.1% for Formula A and 2.3% for Formula B. Although Formula B would release drug at a faster rate than Formula A, as shown in the previously reported dissolution studies (section 3.3), it was clear that total drug release would occur between 7 and 11 hours and that both formulations could be expected to exhibit sustained release properties *in-vivo*.

4.2. MATERIALS AND METHODS

4.2.1. MATERIALS

ß-hydroethyltheophylline, anhydrous (Sigma Chemical Co., U.S.A.)

Caffeine, anhydrous (Sigma Chemical Co., U.S.A.)

Theobromine, crystalline (Sigma Chemical Co., U.S.A.)

Para-xanthine, crystalline (Sigma Chemical Co., U.S.A.)

Theophylline, anhydrous (Sigma Chemical Co., U.S.A.)

Tetrahydrofuran, HPLC grade (BDH Chemical Ltd., England)

Chloroform, HPLC grade (BDH Chemical Ltd., England)

Isopropyl alcohol, HPLC grade (BDH Chemical Ltd., England)

Sodium acetate 3 hydrate, HPLC grade (BDH Chemical Ltd., England)

Glacial acetic acid (BDH Chemical Ltd., England)

4.2.2. *IN-VIVO* STUDY PROTOCOL

After providing written informed consent (Appendix A14), twelve healthy non-smoking male volunteers between the ages of 22 and 38 years and weighing from 50 to 70 kg (Table 3) participated in this study. None were taking any medication or had a history of gastrointestinal disorders. The volunteers were randomly divided into 2 groups and administered the preparations according to the sequence shown below:

Group (6 volunteers/group)	Period		
	I	II	III
1	Formula A	Formula B	solution
2	Formula B	Formula A	solution

The dose of all the preparations was equivalent to 250mg theophylline and the washout period between the treatments was 7 days. The theophylline content of Formula A and B is as shown in Table 4. A weight of each preparation equivalent to 250mg dose was measured and administered in a size 0 hard gelatin capsule (Elanco Qualicaps, Lilly Industries, England) while the volume of the solution was 100ml (2.5mg/ml). In each case, the preparation was administered after an overnight fast with 200 ml of water for Formula A or B, and 100ml in the case of the solution. No food or drink was allowed for at least 2 hours after dosing.

Table 3 : BIODATA OF VOLUNTEERS IN VIVO STUDY I

Volunteer	Age (yrs)	Weight (kg)	Height (cm)
1	22	57.0	172
2	22	50.0	158
3	29	68.0	177
4	24	55.0	165
5	35	52.3	163
6	34	70.4	172
7	25	63.6	175
8	35	65.9	170
9	34	70.4	170
10	38	70.0	175
11	23	52.0	171
12	23	51.0	174

Volunteers recruited from Asian population living in Malaysia and accustomed to eating a local diet.

Table 4 : THEOPHYLLINE CONTENT OF FORMULA A AND FORMULA B

	Weight of pellets (mg)	Weight of theophylline (mg)	Percentage by weight
formula A	400.0	304.1(2.4)	76.1(0.6)
formula B	400.0	307.2(2.6)	76.8(0.7)

Figures in brackets = standard deviation (n=6)

Lunch and supper, comprising chicken with rice, were served at 200 minutes and 560 minutes respectively. Ingestion of alcohol and xanthine-containing food or beverages was prohibited for 24 hours before, during and 36 hours after each drug dosing. Blood samples of 5 ml volume were collected in plain vacutainers at 0 (before dosing), ½, 1, 1½, 2, 3, 4, 6, 8, 10, 14, 18, 24 and 36 hours after dosing for Formula A and B, and in the case of the solution at 0 min, 10 min, 20 min, 40 min, 1, 2, 3, 4, 6, 8, 10, 14, 18 and 24 hours after dosing. An in dwelling cannula was used for drawing the blood during the first 24 hours. The subsequent sample was taken by direct venupuncture. The blood samples were allowed to stand for 2 hours before centrifuging for 10 min at 2000 G. The serum was then transferred to separate glass containers and kept frozen until analysis.

4.2.3. ANALYSIS OF SERUM SAMPLES

Serum levels of theophylline were measured using a reversed phase high performance liquid chromatographic (HPLC) method. The HPLC system consisted of a Gilson model 802 manometric module, a Gilson Holochrome variable wavelength detector and a Gilson model 302 solvent delivery system, equipped with a Shimadzu C-R6A Chromatopac integrator. The column used was a 125mm × 4mm stainless steel cylinder packed with 5µ particle size Lichrosorb RP-18 (Merck) and fitted with a direct connect refillable guard column. The mobile phase comprised 1.5% tetrahydrofuran (THF) in 0.01M sodium acetate buffer adjusted to pH 4.3 with glacial acetic acid. Analysis was run at a flow rate of 1.3 ml/min with the detector operating at 273nm.

Prior to analysis, the drug was extracted from the serum using the following procedure. A 250 µL aliquot of serum sample was accurately measured into an Eppendorf microcentrifuge tube, followed by the addition of 50µL of 2.5mg/100ml ß-hydroxyethyltheophylline (BHET) internal standard solution and 1 ml of 8:2 chloroform-isopropyl alcohol extracting solvent. The mixture was vortexed for 1 min and then centrifuged at 9800 G for 1.5 min. A 0.8ml volume of the supernatant was then removed and dried under a gentle stream of nitrogen at 60°C in a reactivial. The residue was reconstituted with 100µL of mobile phase and a 20µL volume was injected onto the column. All the samples were analyzed in duplicate and average value calculated.

Theophylline standards were prepared by spiking drug-free serum in a concentration range

of 1-8µg/ml. Standard curves, recoveries and precision studies were performed using these serum standards. In addition, detector linearity was determined with theophylline standards prepared in water in the concentration range of 0.5-16µg/ml. The related xanthines, theobromine, para-xanthine and caffeine were injected onto the column under the same analytical conditions to establish the specificity of the method.

4.2.4. DATA ANALYSIS

A model independent numerical deconvolution technique using the trapezoidal formula (*Langenbucher and Moller* 1983) was applied to the serum data to estimate the *in-vivo* dissolution profiles of the preparations. This numerical algorithm requires that the concentration data are entered on each occasion for a set of regular time points which are invariant. Whenever necessary, experimentally measured values of concentration time data were interpolated using the spline and akima methods of Fried and Zeitz (1973). The specific advantage of this interpolation procedure is that the fitted data must pass through all the measured data points. A computer program suitable for effecting the interpolation and the deconvolution procedures was used in the calculation (University of Science, Malaysia). The program was written in Turbo Basic (Borland International, Inc.) and runs on a desktop computer. In addition to the interpolation capability, it also permits the data points to be generated from pharmacokinetic parameter values which can be estimated by curve fitting of the raw serum data using a non-linear least square fitting method. The flow-charts of the program are given in Appendix A18.

The serum data were also analyzed using the parameters, total area under the serum level curve (AUC_o^α), maximum serum concentration (Cmax), time to reach maximum serum concentration (Tmax) and lag-time. The first parameter was calculated using the trapezoidal formula, whilst Cmax and Tmax were obtained directly from the serum values (*Weiner* 1981). The lag time of absorption was estimated when necessary, by extrapolating from the serum level curves.

Individual pharmacokinetic parameters of the volunteers were also estimated by curve fitting the serum data of the theophylline solution using the AUTOAN - NONLIN computer program described by Wagner (1975a). The numerical values are given in Appendix 19. Satisfactory fits were obtained with a one-compartment pharmacokinetic model with first order input (ka)

and elimination (ke), as evidenced by the small standard deviations of individual parameter values generated by the program (Appendix 19). However, fitting the serum data of the sustained release formulations is more complex. Whilst in general, a first order input process can be assumed for a drug solution, the absorption from a sustained release preparation may not be readily described by a simple first order or zero order rate. Because the drug dissolution of a sustained release product occurs over an extended period of time, factors such as gastric emptying, gastrointestinal transit and interactions with food may further complicate the absorption process. This is clearly illustrated in the results in the next section (Chapter 5). The absorption from the sustained release products appears to be biphasic or triphasic in nature, and could be modified by the presence of food.

In the curve fitting procedure, the sums of squares of the difference between each observed and model calculated value are minimized. Therefore, if an inappropiate input process is assumed, it is possible to obtain an unusual combination of parameter values that best fit the data, but which may not be the true best fit (*Jelliffe* 1986). Under this condition, the accuracy of the other parameter values, for example that of the ke, may be affected. In view of all these, the serum data of the sustained release products were not analysed using this fitting procedure.

In the computation of the AUC_0^∞ values, the terminal area under the curve (after the last sampling point) was estimated by dividing the last serum concentration with the ke (*Gibaldi and Perrier* 1982). Because of the reasons mentioned above, the ke values were not estimated from curve fitting of the serum data of the sustained release preparations. Instead, the values obtained from the solution data were used with the assumption that they were unchanged between administration of the preparations. Although the ke values can be estimated with sufficient accuracy from the terminal slope of the serum level curves (assuming absorption is complete) by logarithmic conversion of the data and application of linear regression (*Gibaldi and Perrier* 1982a), this was not carried out because of the large interval (12 hours) between the last two time points.

4.3 RESULTS AND DISCUSSION

4.3.1 ANALYSIS OF SERUM THEOPHYLLINE CONCENTRATION

To be adequate for this study, the HPLC method must be sufficiently sensitive to detect the theophylline concentration over the duration of the study. At the same time, it must be specific enough to distinguish the parent drug and potentially interfering methylxanthines and their metabolites, namely caffeine, theobromine and paraxanthine (*Lewis and Johnson* 1978). Orcutt *et al* (1977) described a HPLC method for the analysis of theophylline in which serum samples containing theophylline were injected directly onto a μBondapak C_{18} column after precipitating the serum proteins with an equal volume of a 20% acetonitrile solution. Moreover, these authors reported that sole interference from a substance which they presumed to be paraxanthine could be obviated by adjusting the composition of their mobile phase from 7 parts per hundred of acetonitrile to 5 parts per hundred in 0.01M acetate buffer. Because of its apparent simplicity and specificity, this method was evaluated for use in this study. Initially, it was found that the separation of the paraxanthine and theophylline peaks was unsatisfactory. Therefore a modification was made in which tetrahydrofuran was substituted for acetonitrile in the acetate buffer (*Hartley et al* 1984; *Grgurinovich* 1986). It may be seen in Figure 21, that a mobile phase consisting of 1.5% tetrahydrofuran in 0.01M acetate buffer produced a chromatogram of the required specificity.

A further modification of the method was to include a solvent extraction step. This has the advantage of increased specificity, whilst the evaporation and reconstitution of the residue in a minimal amount of the mobile phase adds to the sensitivity of the method (*Van Aerde et al* 1981). Moreover, the cleaner injection material provided by the extraction procedure, will ensure a longer column life (*Gerson and Anhalt* 1980). A mixture of chloroform and isopropyl alcohol has been shown to be an efficient extractant for theophylline and BHET (*Adams et al* 1976; *Van Aerde et al* 1981). Recovery values of greater than 90% were obtained for both compounds when a 8:2 mixture of the two solvents (chloroform : isopropyl alcohol) was used in the present study. The numerical values are presented in Table 5.

Figure 22 shows the chromatograms of serum samples taken from a volunteer before and 10 hours after dosing of Formula A. The blank sample (before dosing) is clean and devoid of

Figure 21 : CHROMATOGRAM OF THEOPHYLLINE AND RELATED XANTHINES

1. theobromine
2. para-xanthine
3. theophylline
4. BHET
5. caffeine

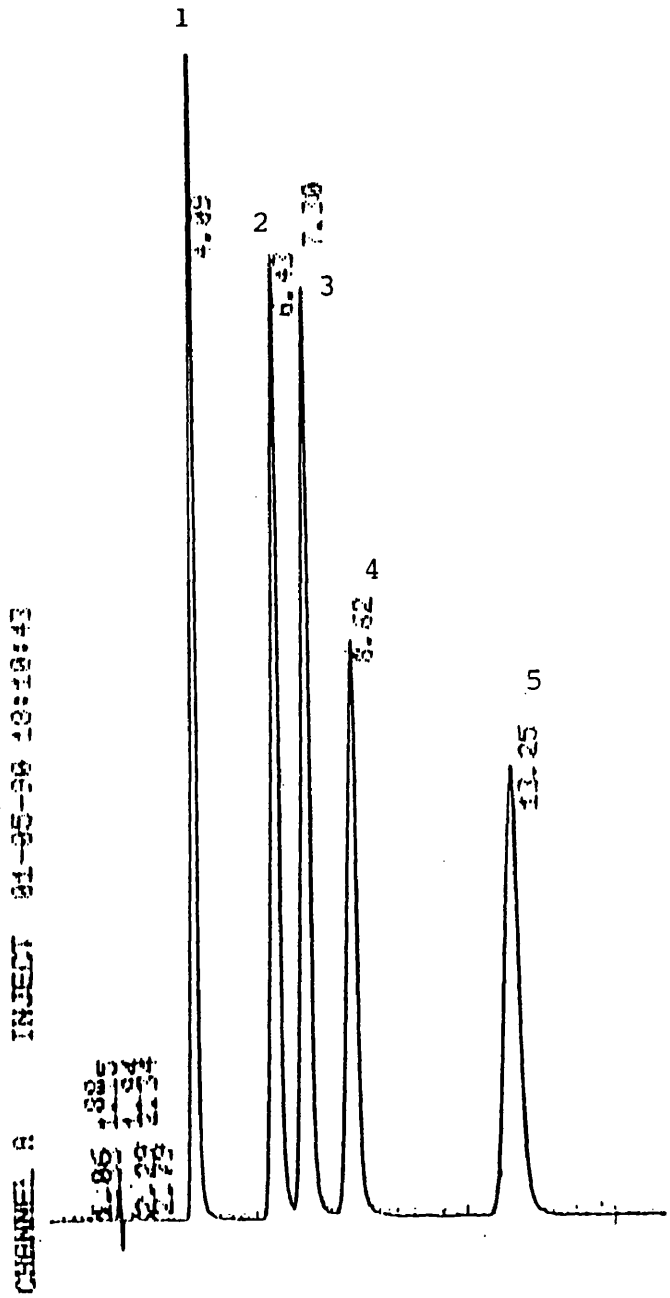


Table 5 : PRECISION AND RECOVERY DATA OF HPLC METHOD (IN VIVO STUDY I)

Recovery of theophylline and BHET

Conc.(ug/ml)	1.0	4.0	8.0	BHET (n=12)
%	94.70	93.52	94.80	93.12
c.v.	1.29	2.12	2.05	2.21

c.v. = coefficient of variation (n=6)

Precision of theophylline assay at three levels of concentration

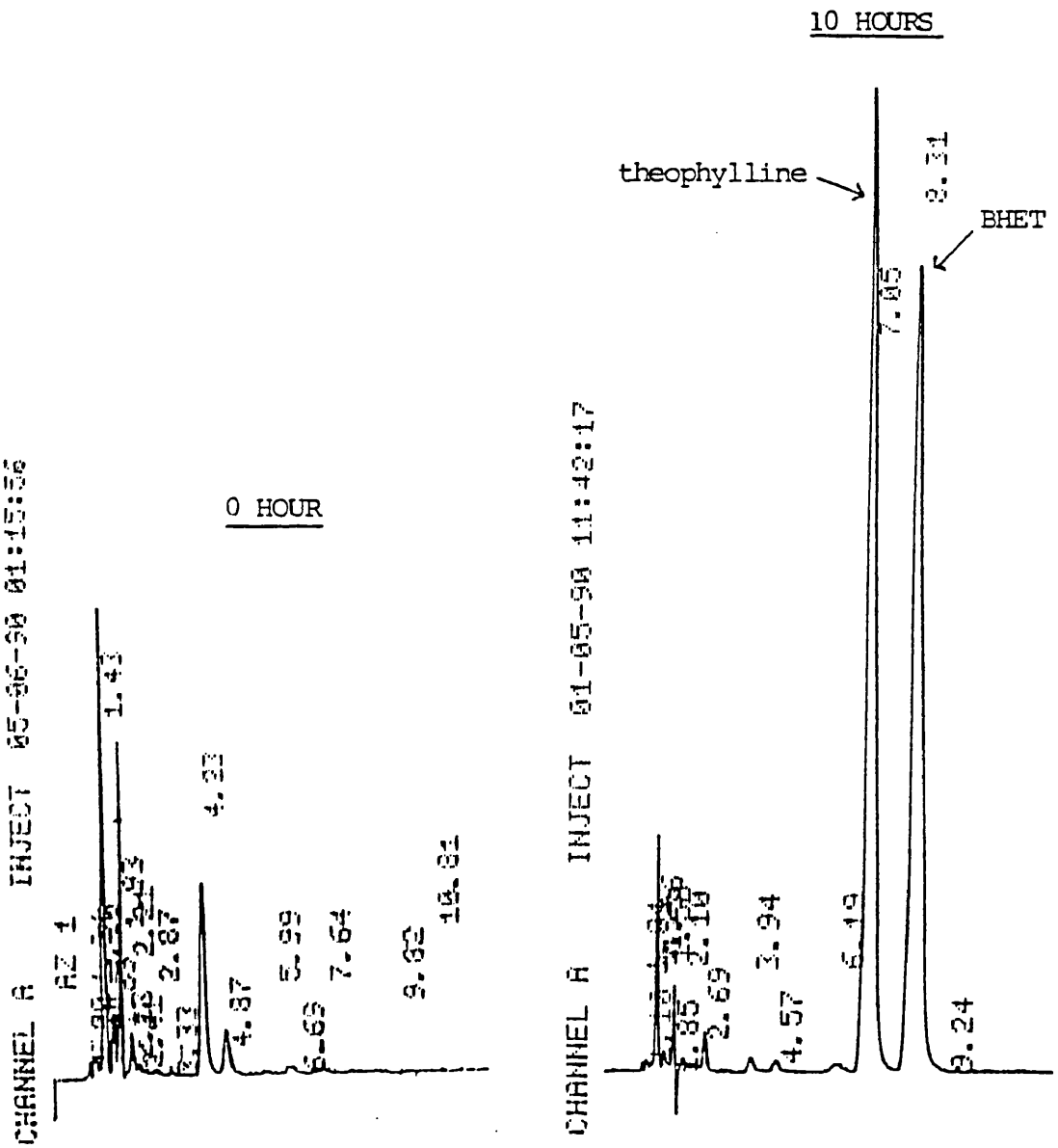
Conc.(ug/ml)	c.v. within day	c.v. between day
2	3.58	4.26
4	2.48	4.80
8	3.83	7.12

c.v. = coefficient of variation (n=6)

Detection limit

0.1 ug/ml signal to noise ratio > 6

Figure 22 : CHROMATOGRAM OF SERUM SAMPLES TAKEN FROM A VOLUNTEER AT 0 HOUR AND 10 HOURS AFTER ADMINISTRATION OF FORMULA A



interference at the retention times of theophylline and BHET. In the case of the 10 hour sample in which BHET was added, the peaks of the two compounds are sharp and well separated, with no shoulder or tailing observed. Chromatographic time is short, being less than 10 minutes. This is an important advantage considering the number of serum samples that need to be analyzed in duplicate. Using spiked serum samples, the precision of the method was evaluated. Satisfactory coefficients of variation were obtained for the three concentration levels of theophylline, both for within day and between day assays. These values are given in Table 5. Furthermore, over the range of concentrations determined, the detector response and standard curve are both linear (Figures 23,24).

4.3.2 IN-VIVO PERFORMANCE OF NOVEL PREPARATIONS AND CORRELATION OF IN-VIVO/IN-VITRO RESULTS

The results obtained from the analysis of the serum samples are tabulated in Appendix A15 and the average serum levels for the three preparations are as presented in Figure 25. A qualitative difference is clearly evident. Theophylline administered as a solution is rapidly absorbed achieving a peak concentration within an hour after dosing. In contrast, Formula A and Formula B both exhibit serum profiles that are reflective of a slow and sustained rate of absorption. Peak concentrations are achieved at approximately 10 and 7 hours respectively, suggesting that the absorption rate of Formula A is relatively slower.

The *in-vivo* release profiles of the two preparations calculated using the deconvolution procedure are plotted in Figure 26 together with the *in-vitro* results. It is apparent from the plots that both preparations show satisfactory *in-vivo* sustained release behaviour extending over more than 14 hours in the case of Formula B and 18 hours in Formula A. Whilst the release rate of Formula A is slower than that of B, both preparations however, achieve essentially complete dissolution. After correcting for lag times where appropriate, individual values of the time for 50% of the dose to be released and the fraction of dose released were calculated and are reported in Table 6. These two parameters were chosen for comparing the respective rate and extent of release between the two preparations. The difference in the *in-vivo* rate of release was found to be statistically significant ($P < 0.001$) when analyzed using an analysis of variance procedure (ANOVA) appropriate for the crossover design (*Wagner* 1975b). However the extent of release was not significantly different ($P = 0.499$).

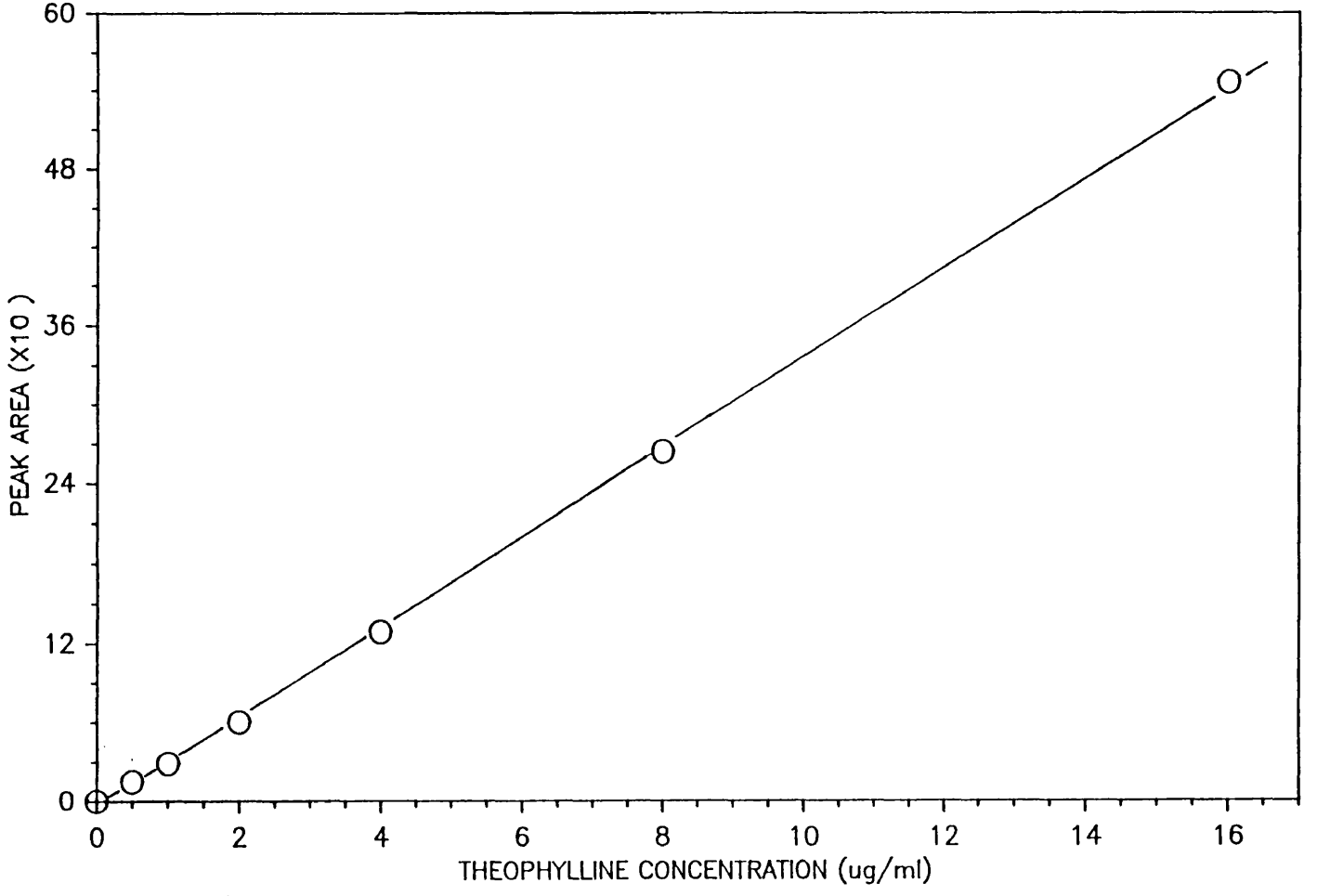


FIGURE 23: PLOT OF THEOPHYLLINE CONCENTRATION VERSUS PEAK AREA SHOWING DETECTOR LINEARITY OF HPLC ANALYSIS (STUDY 1)

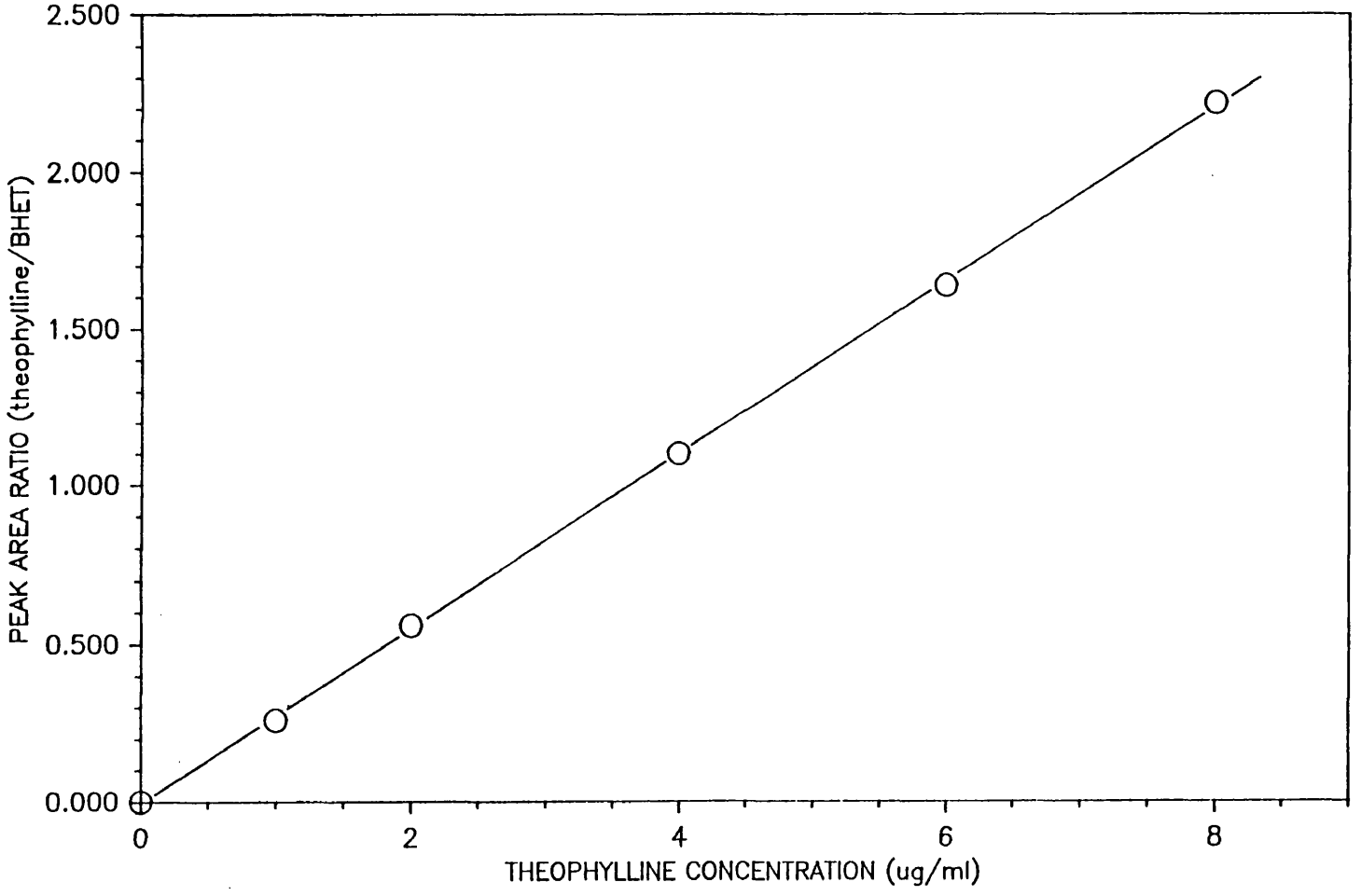


FIGURE 24: STANDARD CURVE OF HPLC ANALYSIS (STUDY 1)

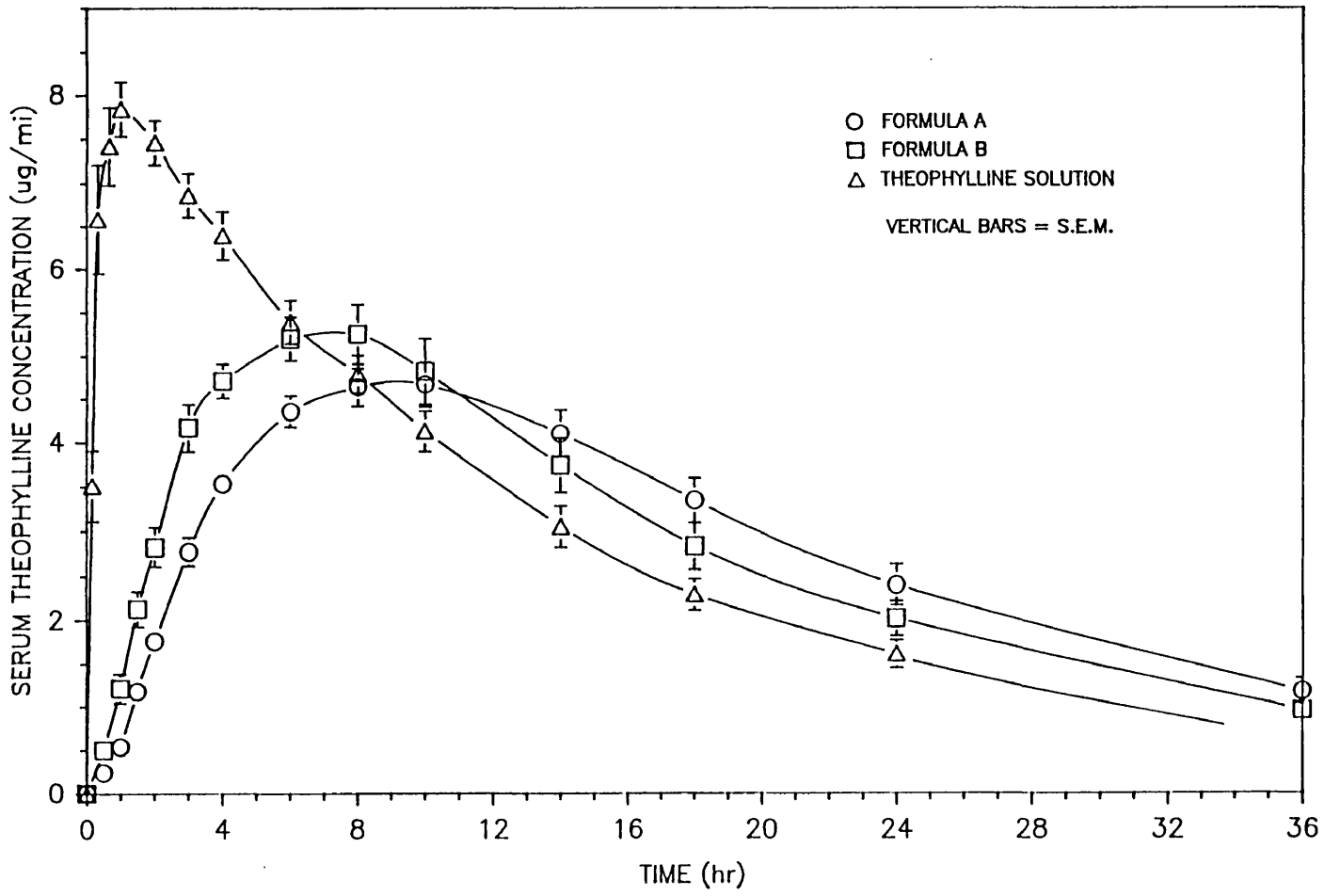


FIGURE 25: AVERAGE SERUM THEOPHYLLINE CONCENTRATION PROFILES OF THEOPHYLLINE SOLUTION, FORMULA A AND FORMULA B (DOSE = 250 mg, N = 12)

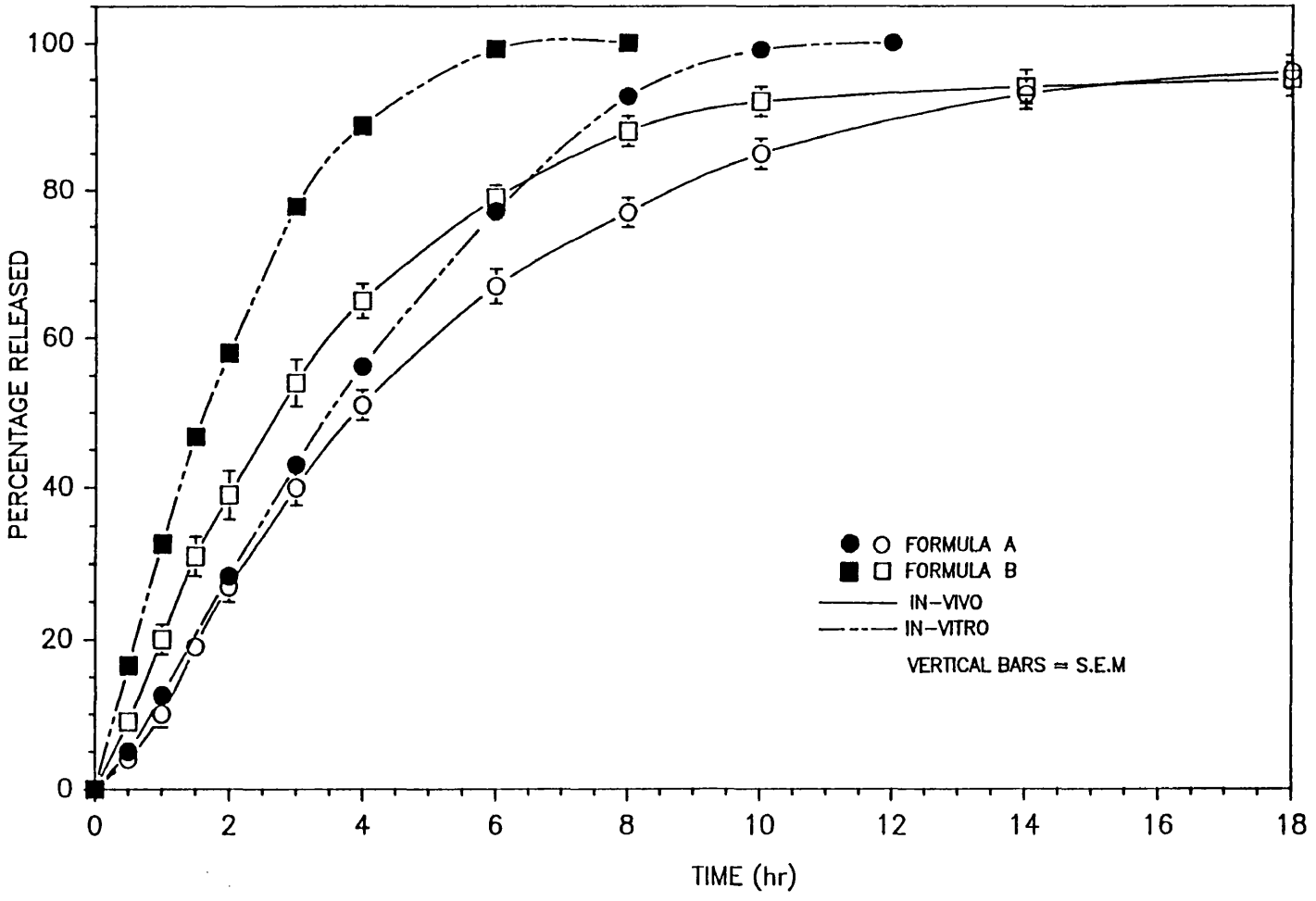


FIGURE 26: IN-VITRO AND IN-VIVO THEOPHYLLINE RELEASE FROM FORMULA A AND FORMULA B

Table 6 : NUMERICAL VALUES OF T50% AND FRACTION OF DOSE RELEASED IN VIVO OBTAINED WITH FORMULA A AND FORMULA B

Volunteer	T50%		Fraction of dose released	
	Formula A	Formula B	Formula A	Formula B
1	4.6	2.0	0.83	0.89
2	2.9	2.4	0.97	1.12
3	3.2	1.7	1.24	1.18
4	3.5	2.2	1.03	0.87
5	5.1	3.4	1.01	0.94
6	3.2	2.6	0.90	0.90
7	3.4	1.6	0.87	0.99
8	3.6	2.2	1.00	1.03
9	3.0	2.2	0.96	0.90
10	3.2	3.1	1.06	0.93
11	3.2	1.8	1.02	0.97
12	4.2	2.9	0.97	0.90
Mean	3.6	2.3	0.99	0.97
SEM	0.2	0.2	0.03	0.03
	P < 0.001		P = 0.4998 (NS)	

Inspection of the plots shown in Figure 26 reveals an association between the *in-vitro* and *in-vivo* results. Formula A, which has a slower *in-vitro* dissolution, also exhibited a slower *in-vivo* release. Therefore the results of the *in-vitro* test can be used to compare the *in-vivo* performance of these preparations. It is also noted that the *in-vivo* release, is on the whole slower than that achieved *in-vitro*. For the two preparations, the ratio

$$\frac{T_{50\%}\ \textit{in-vivo}\ \text{dissolution}}{T_{50\%}\ \textit{in-vitro}\ \text{dissolution}}$$

has an average value of the order 1.4.

This is qualitatively in accord with the findings of Beckett *et al* (1980) and Benedikt *et al* (1988).

A number of approaches have been used for interrelating *in-vivo* and *in-vitro* data. Some of these try to relate single-point measurements, such as area under the blood concentration curve values, peak concentrations or peak times, to the *in-vitro* times for 50% drug release or other *in-vitro* parameters (*Lordi* 1986). However, considering the amount of information contained in these time functions and the complexity of the processes involved, this kind of correlation is considered rather rudimentary (*Langenbucher and Moller* 1983). Such univariate single point representations can obscure much of the information otherwise available from dissolution and blood level profiles. Methods based on statistical moments analysis have also been used (*Moller and Langenbucher* 1982; *Banaker and Block* 1983), but likewise, they do not provide information regarding the time course of the drug release or absorption (*Chan et al* 1987). By comparison, the deconvolution method used in the present study permits the time course of these processes to be estimated. An interesting feature of this method is that, depending on whether solution data or intravenous data are used as the weighting function, the *in-vivo* release is calculated alone or as a combined process with absorption. In the latter case, the results of the analysis will be similar to that obtained using the Wagner-Nelson (1964) or Loo-Riegelman (1968) methods for the one and two compartment pharmacokinetic models respectively (*Langenbucher* 1982). In another approach (which is the opposite of the deconvolution procedure) the *in-vitro* dissolution profile is transformed into a predicted *in-vivo* response using the operation of convolution (*Tuey* 1984). The weighting function in this case is determined from a deconvolution of the *in-vitro* release profile and blood levels of a reference product, which is similar to the dosage form undergoing testing.

The numerical deconvolution method used in the present study is noncompartmental in nature, and makes no prior assumption of the input kinetics. The only assumption made is the linear and time-invariant behaviour of the system. The algorithm has been shown to be accurate and does not create error during calculation (*Chan et al,* 1987). However, a disadvantage with the algorithm is its inherent tendency towards instability when the quality of the raw data is poor. Smoothing or fitting of the raw data were suggested for overcoming this problem (*Langenbucher and Moller,* 1983). Computational instability was encountered with some of the data sets in the present study but were readily resolved by smoothing some points in either the weighting data alone, or both the weighting and response data. This was carried out by manually plotting the raw values on graph paper.

The results in Figure 27 show the plots of the % *in-vivo* versus % *in-vitro* release at times t and t/k respectively, where k is an intensity factor calculated from the ratio of the *in-vivo* over the *in-vitro* T50% released (*Swarbrick* ,1970). The advantage of using the factor k is in obtaining a slope of near unity, so that both *in-vivo/in-vitro* release profiles will be equally represented in the plots. It is seen in Figure 27 that the plots of the two preparations are not coincident. This may be attributed to the *in-vitro* test being slightly over sensitive in detecting a larger relative difference between the release rates of the two preparations compared to that observed *in-vivo*. Nevertheless, a good relationship appears to exist between the *in-vivo* and *in-vitro* dissolution rates, albeit the plot for Formula A deviates slightly from linearity. The correlation coefficient for the plot of Formula A is 0.9980 ($P < 0.001$) and for B is 0.9991 ($P < 0.001$).

Figure 28 shows the plots relating the *in-vivo* and *in-vitro* dissolution times. Again the slope of the plots appears different, but the *in-vivo/in-vitro* relationship is fairly linear over a majority of the points, representing about 70% of drug release (each time point shows a 10% increment in percentage released). The correlation coefficients are 0.9842 ($P < 0.001$) and 0.9930 ($P < 0.001$) for Formula A and B respectively. The deviation of the last 2 points in each case is merely a reflection of the more rapid decline in the *in-vivo* dissolution rates after about 60-70% of the dose have been released, compared to the *in-vitro* dissolution. Presumably at this stage, the preparations have reached some parts of the gastrointestinal tract such as the caecum, where changes in agitation intensity or other local environmental factors caused the rate of dissolution to decline more rapidly. Such factors are not accounted for in

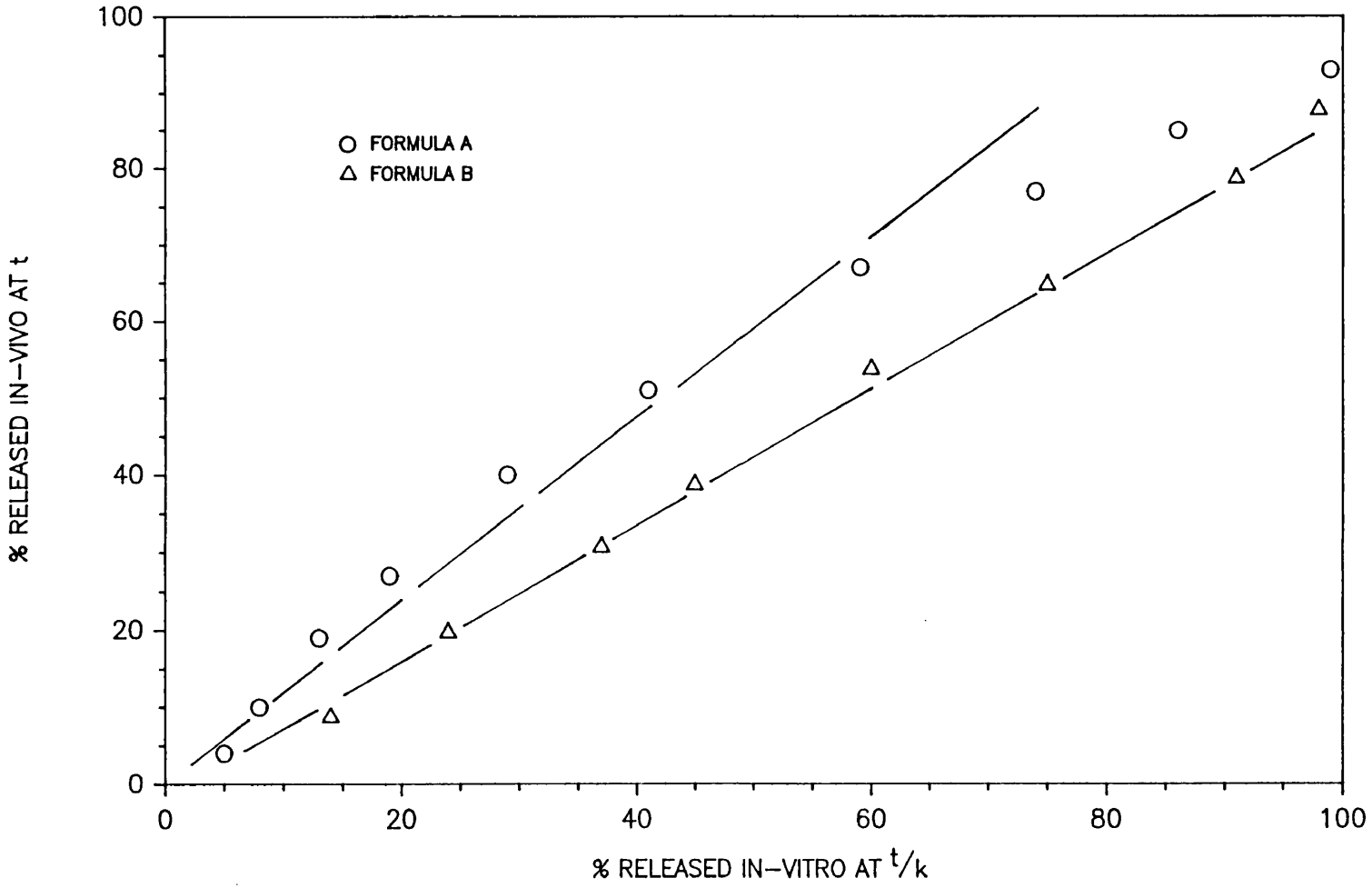


FIGURE 27: PLOT OF PERCENTAGE RELEASED IN-VIVO AT TIME t VERSUS PERCENTAGE RELEASED IN-VITRO AT TIME t/k FOR FORMULA A AND B

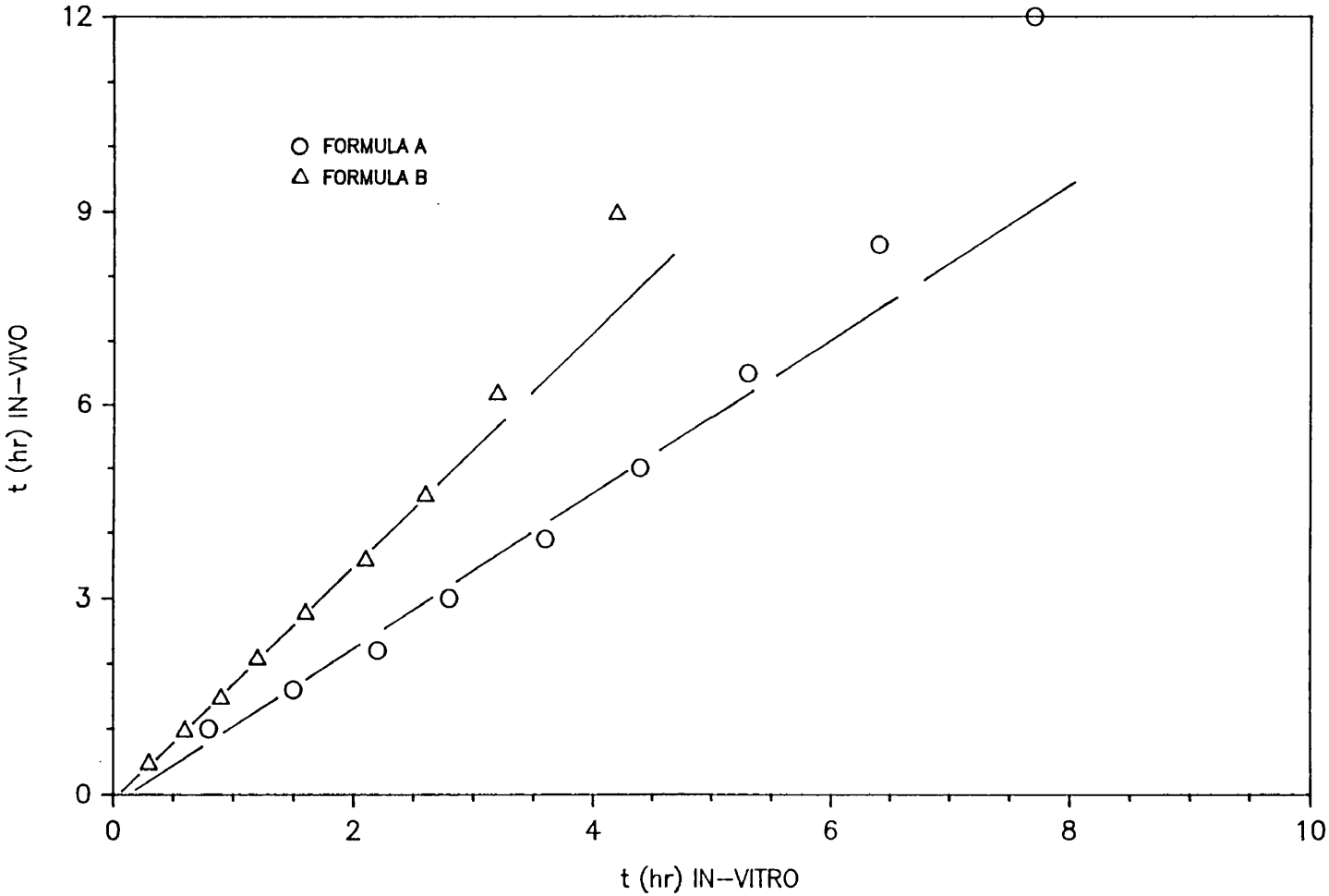


FIGURE 28: PLOT OF TIME OF RELEASE IN-VIVO VERSUS TIME FOR EQUAL PERCENTAGES RELEASED IN-VITRO FOR FORMULA A AND B

the *in-vitro* dissolution test methodology. Despite the shortcomings however, the results above show that a fairly good association can be established between the *in-vivo* and *in-vitro* results.

Individual values of the parameters AUC_o^∞, Tmax, Cmax, lag-time and the elimination rate constant, ke are as shown in Table 7. A lag-time was observed in many of the serum concentration curves. Therefore, the Tmax values presented in the table have been corrected by subtracting the lag-time. The ke values shown in the table were estimated from the serum levels obtained from administration of the theophylline solution. An average ke value of 0.08 hr^{-1} was obtained, equivalent to a biological half-life of about 8.9 hours. This is comparable to the values reported by Hendeles and Weinberger (1982) and West *et al* (1990) for healthy non-smoking adults.

When the parameters were analyzed statistically using the ANOVA procedure mentioned earlier, a statistical difference was obtained between the values of the lag-time ($P = 0.005$), Tmax ($P = 0.001$) and Cmax ($P < 0.001$) but not those of the AUC_o^∞ ($P = 0.305$) (Table 7). Since Tmax and AUC_o^∞ are related to the respective rate and extent of absorption, while Cmax is related to both processes (*Grahnen* 1984), these findings are consistent and in support of the statistical results obtained with the parameters T50% and fraction released, derived from the deconvolution procedure. Therefore it can be concluded that Formula A and Formula B possess comparable extent of *in-vivo* release/absorption but differ in the rate of these processes, with Formula A being slower. The results above also show that Formula A has a significantly longer lag-time than B.

Certain assumptions are involved when using statistical tests such as the ANOVA procedure. For example, it is assumed that the observations are normally distributed and the sample variances homogenous. However, it has also been pointed out that ANOVA is robust with respect to violation of many of the assumptions (*Kirk* 1968), and there is seldom a need for data transformation (*Healy et al* ,1986). In the present analysis, the sample variances were evaluated for homogeneity using the Fmax test (*Hartley* 1950), and were all found to conform with the assumption.

Table 7 : NUMERICAL VALUES OF PHARMACOKINETIC PARAMETERS OBTAINED WITH FORMULA A AND FORMULA B

Volunteer	Ke(hr^{-1})	Lag time(hr)		t_{max}(hr)		AUC_o^{∞}(ug hr ml^{-1})		C_{max}(ug ml^{-1})	
	Theophylline Solution	Formula A	Formula B	Formula A	Formula B	Formula A	Formula B	Formula A	Formula B
1	0.11	0.3	0.3	7.7	3.7	61.4	71.5	3.83	4.69
2	0.08	1.0	0.7	9.0	7.3	158.3	161.7	6.81	7.85
3	0.08	0.8	0.5	7.2	5.5	134.6	127.8	5.09	5.48
4	0.08	0.4	0.0	7.6	6.0	115.4	98.0	4.82	5.24
5	0.06	0.9	0.5	13.1	7.5	170.7	151.3	5.68	6.71
6	0.06	0.4	0.3	7.6	5.7	97.9	98.7	3.80	4.23
7	0.05	0.4	0.0	9.6	6.0	138.6	154.0	5.29	6.66
8	0.07	0.0	0.0	10.0	10.0	144.3	151.4	4.85	5.34
9	0.08	0.4	0.4	7.6	6.6	110.1	106.0	4.72	4.91
10	0.10	0.8	0.1	9.2	7.9	99.0	88.9	4.22	4.40
11	0.08	0.0	0.0	10.0	6.0	122.9	108.5	4.82	5.16
12	0.11	0.5	0.0	7.5	7.0	93.3	85.4	3.96	4.57
Mean	0.08	0.5	0.2	8.8	6.6	120.5	116.9	4.82	5.44
SEM	(< 0.01)	0.1	0.1	0.5	0.4	8.8	8.9	0.25	0.31
		P = 0.005		P = 0.001		P = 0.305		P < 0.001	

The AUC_o^∞ values of the two preparations were further compared with the values obtained from the solution data (Appendix 19). An average of 102 ± 14% was obtained for Formula A and 99 ± 12% for B, indicating that both preparations attained complete drug availability since theophylline administered as a solution is completely absorbed (*Hendeles et al* 1977a; *Weinberger et al* 1978). This can also be interpreted to exclude any saturable first-pass metabolism effect on the drug absorption from the sustained release preparations. If indeed this process is operative, the bioavailability as measured from the serum concentration profiles will tend to be lower with the slow release preparations (*Wagner* 1988).

4.4 CONCLUSION

On the basis of the results obtained from this study, it can be concluded that the sustained release theophylline preparations possess satisfactory *in-vivo* performance. The *in-vivo* drug release, particularly that of Formula A, is adequately sustained producing a satisfactory serum concentration profile over 24 hours. The contained drug is completely released and absorbed. In addition, the *in-vivo* drug release rate can be effectively varied by manipulating the thickness of the film coat which is also reflected in the results of the *in-vitro* studies. In this regard, a good association can be established between the *in-vivo* and *in-vitro* data.

Chapter 5: COMPARATIVE BIOAVAILABILITY AND GASTROINTESTINAL TRANSIT STUDIES (STUDY II)

5.1 INTRODUCTION

The importance of studying the gastrointestinal transit properties and the effects of food status in the development of a good sustained release product has been discussed previously (Chapter 1). Information gathered from such studies can aid in optimising the dosage form design and also ensure that the product meets the objectives of providing a consistent and predictable *in-vivo* performance. In view of such considerations, a study was now conducted to evaluate the effects of food status (fed and fasted) on the absorption and gastrointestinal transit properties of the novel sustained release theophylline formulation in healthy human volunteers. In addition, its bioavailability was also compared with a commercial theophylline product, Uniphyllin Continus Tablets (Napp Laboratories, U.K.), which is commonly used in the United Kingdom. This product is available as a single unit sustained release tablet containing either 300mg or 400mg of theophylline.

Formula A which produced a more satisfactory serum level profile in the previous *in-vivo* study was chosen for the present evaluation, whilst the 300mg tablet of Uniphyllin was used for comparison because of its lower dosage. Transit of the dosage form in the gastrointestinal tract was determined using a gamma scintigraphic method with Technetium-99m (99m-Tc) as the radiolabel. An equal amount of inert lactose pellets of similar size and density containing 5% Amberlite (labelled with 99m-Tc) was administered together with the theophylline pellets to act as the radioactive marker for imaging. This was necessary because the radiolabelling procedure might affect the drug release properties of the theophylline pellets. The contents of the Amberlite pellets are as shown in Table 1. Incorporation of a small amount (5%) of the anionic ion exchange resin (Amberlite CG400) has been shown to produce a stable and satisfactory labelling with the radionuclide 99m-Tc (*Devereux* 1987; *Clarke* 1989). However, the gastrointestinal transit of the Uniphyllin tablet was not monitored because it is a pre-prepared commercial product.

5.2 MATERIALS AND METHOD

5.2.1 MATERIALS

Uniphyllin Continus Tablets 300mg (Napp Laboratories Ltd, U.K.) - manufacturing date: May 1989 (B.N. 10)

Other materials used were as listed in section 4.2.1

5.2.2 PREPARATION AND COATING OF 5% AMBERLITE PELLETS

The 5% Amberlite pellets were prepared as described in section 2.2.2. Only pellets of size fraction similar to the theophylline pellets (1.18-1.4mm sieve) were used. They were coated using the aqueous colloidal dispersion of ethylcellulose (Ethocel AQ) with a film thickness of TWG 5.8%. For easier radiolabelling, methylcellulose (viscosity grade 15cp) was incorporated into the ethylcellulose coat to increase the permeability of the film. The ratio of methylcellulose to ethylcellulose used was 0.4. Coating was performed using the fluidized bed coating technique described in section 3.2.2. After coating, the pellets were cured for 24 hours at 60°C in an oven.

5.2.3 DENSITY DETERMINATION OF PELLETS

The density of the coated Amberlite pellets and the theophylline pellets used in this part of the study was determined using the Beckmann Air Comparison Pycnometer (Model 930). For each type of pellets, the measurement was repeated 3 times and the average value calculated.

5.2.4 RADIOLABELLING OF AMBERLITE PELLETS WITH 99m-Tc

The radionuclide 99m-Tc was eluted daily from a commercial generator (Elumatic III, CIS France). A sterile pyrogen free solution of sodium pertechnetate ($Na^+[^{99m}Tc]O_4^-$) in normal saline was obtained from the elution. The film coated Amberlite pellets (400mg) were soaked in 3ml of the 99m-Tc solution containing approximately 200MBq of activity. After half an hour of soaking, the solution was removed and the pellets washed twice with 5ml of normal saline solution. They were then soaked for another hour in 10ml of normal saline whilst rotating on a vertical gantry at 10rpm in a shielded water bath (Townson and Mercer Ltd, Surrey) at 37°C. This was to ensure that the unbound radiolabel was rinsed off from the surface of the pellets. The solution was removed and the pellets dried, overnight, in an oven at 50°C. The following morning, the pellets were weighed and assayed for 99m-Tc activity.

All assays of the 99m-Tc activity (above 1MBq) were performed using an isotope assay ionisation chamber (Pitman Instrument, Surrey Model 270).

5.2.5 *IN-VITRO* TESTING OF 99m-Tc BINDING TO AMBERLITE PELLETS

Six batches of pellets weighing 400mg each, were radiolabelled as described in section 5.2.4. After labelling, each batch was placed in separate glass tubes containing 20ml of simulated gastric juice (USP XX 1980, omitting pepsin), of pH=1.2. The tubes were sealed and placed on a vertical gantry rotating at 10rpm in the shielded water bath at 37°C. After 4 hours, the pellets were removed and the remaining simulated gastric juice in each glass tube was assayed for 99m-Tc activity. The radioactivity released into the supernatant was calculated as a percentage of the initial activity after correcting for decay. The experiment was repeated with phosphate buffer B.P. of pH=4 and simulated intestinal buffer (USP XX 1980, omitting pancreatin) of pH=7.5. However, in these two experiments, the radioactivity released into the supernatant was determined after the pellets had been soaked in the fluids for 14 hours rather than 4 hours because it was felt that this would approximate the *in-vivo* transit time of the pellets to reach the lower part of the gastrointestinal tract (colon). The integrity of the pellets was also visually inspected during the course of the studies.

The pellets were further examined for distribution of radioactivity within each capsule. Two batches of pellets weighing 400mg each were radiolabelled as described above. After drying for 4 hours at 50°C in an oven, each batch was divided into 10 portions. Each portion was weighed and its radioactive level was determined. The percentage deviation of radioactivity of each sample from the mean radioactivity of all the samples was calculated, after correction for weight variations.

5.2.6 *IN-VITRO* DISSOLUTION STUDIES OF UNIPHYLLIN TABLETS

The dissolution profiles of the Uniphyllin tablets in pH 1, 4 and 7 were determined using the method mentioned in section 2.2.3.

5.2.7 *IN-VIVO* STUDY PROTOCOL

Study Design

Twelve non-smoking male volunteers between the ages of 19 and 24 years and weighing from

56 to 83 kg (Table 8), participated in the study after providing written informed consent (Appendix 20). All declared themselves healthy, were not taking any medication and had no history of gastrointestinal disorders. The volunteers were randomly divided into 2 groups of 6 volunteers each and administered the preparations according to the study design shown below:

	Formula A	Uniphyllin
Dosed fasted	Group 1	Group 1
Dosed fed	Group 2	Group 2

Each volunteer in each group was randomised to receive either Formula A or Uniphyllin on the first occasion. After a one week washout period, each volunteer then received the other preparation. The dose of Formula A was equivalent to 300mg theophylline and was administered together with an equivalent weight (395mg) of the 5% Amberlite pellets, labelled with 7.4MBq of activity, in a size 000 hard gelatin capsule (Elanco Qualicaps, Lilly Industries, England). The Uniphyllin preparation was administered as a single tablet containing a labelled dose of 300mg theophylline. Both preparations were taken with 150ml of water.

Food Intake

The first group of volunteers was dosed after an overnight fast. For the second group, the preparations were taken immediately after a standard breakfast comprising 23G cornflakes with 230ml semi-skimmed milk and 2 pieces of white bread spread with 15G butter and 20G marmalade. The breakfast is similar to the meal used by Devereux (1987) and has a caloric value of 535kcal. At 200 mins after dosing, all volunteers were given a standard lunch of a MacDonalds' Quaterpounder hamburger, french fries (medium), an apple and a cup of orange juice. Supper, consisting of chicken with rice was provided at 560 mins after dosing. Ingestion of alcohol and xanthine-containing food or beverages was prohibited for 24 hours before, during and 36 hours after commencement of the study.

Blood Sampling

Blood samples of 5ml volume were collected in plain vacutainers at 0 (before dosing), ½, 1,

Table 8 : BIODATA OF VOLUNTEERS IN VIVO STUDY II

Volunteer	Age (yrs)	Weight (kg)	Height (cm)
KI	21	82.7	180
JE	21	56.8	175
LM	21	56.8	172
KN	19	60.0	165
NA	20	56.8	165
SL	22	70.0	173
EY	20	56.8	168
AM	20	77.3	175
VK	22	75.0	173
WI	22	72.7	178
JA	24	67.3	175
WW	22	69.1	178

Volunteers recruited from Asian population living in England and accustomed to eating a western diet.

2, 3, 4, 6, 8, 10, 14, 18, 24, 30 and 36 hours after dosing. An in-dwelling cannula was used for withdrawal of blood during the first 24 hours. Subsequent samples were taken by direct venupuncture. The blood samples were allowed to stand for 2 hours before centrifuging for 10 min at 2000G. The serum was then transferred to separate glass containers and kept frozen until analysis.

Gamma Scintigraphy

Imaging of the pellets in the gastrointestinal tract was performed using a Siemens Rota gamma camera system with two opposed ZLC 37-tube detectors, each having a 40cm field of view and capable of simultaneous data acquisition. Both detectors were fitted with a low energy parallel hole collimator suitable for 99m-Tc imaging. An on-line ADAC digital computer (DPS 3300) was connected to the camera for data processing. The geometric mean response of the camera system has been assessed and validated by Devereux (1987) using a point source of 99m-Tc, and found to be satisfactory.

During the study, the volunteer was seated comfortably between the two heads of the camera in an upright position by leaning against the posterior detector. When the preparation has been swallowed, images of 60s per frame were immediately acquired continuously and simultaneously from the anterior and posterior detectors for 90 mins. Thereafter, images were acquired at 10-15 min interval. If necessary, more frequent acquisition was made to maximise the information collected over the critical period of gastric emptying. During this time, the volunteer was allowed to move away from the camera in between imaging. The volunteer had a small sealed source of 0.6MBq 99m-Tc firmly taped to the skin at the position of his lower costal margin for repositioning when the images were taken. The source was also used as an anatomical reference marker. After 6 hours of imaging, the acquisition time of each image was increased to 120s per frame, to compensate for the radioactive decay of the 99m-Tc. The imaging was continued until 12 hours after dosing and the data collected were stored on hard disk for processing and on magnetic tape for archiving. All volunteers were also asked to report any bowel movements during the course of the study.

5.2.8 ANALYSIS OF SERUM SAMPLES

Serum levels of theophylline were measured using the HPLC method described in section 4.2.3, but with changes in the detector and integrator used. The HPLC system employed in

the present analysis was equipped with a Waters Lambda-Max Model 481 variable wavelength detector and Philips Pye Unicam PU 4810 integrator.

Because of the changes made in the system hardware, particularly the detector, it was felt necessary to repeat the evaluation of the analytical method performed previously. They included determination of the detection limit, precision and linearity of detector response and standard curve.

5.2.9 ANALYSIS OF SERUM DATA

The serum concentration data were analyzed using the Wagner-Nelson method (1964) to estimate the percentage absorbed versus time profiles of each preparation administered under fed and fasted conditions. The method is based on a one-compartment pharmacokinetic model and is appropriate for theophylline, since its kinetic analysis can be satisfactorily applied using this model (*Loughnan et al* 1976; *Mungall* 1983). Satisfactory results were obtained from fitting the serum data of the theophylline solution to this model in the previous section (4.2.4). The ke values (elimination rate constant) required in the calculation using the Wagner-Nelson equation were estimated from the terminal slope of individual serum level curves. For reasons mentioned in section 4.2.4, the ke values were not estimated by curve fitting of the serum data. Results of the previous in-vivo study in section 4, also indicated that the in-vivo release/absorption of Formula A was complete by 24 hours after dosing. As for Uniphyllin, its serum profiles shown in Figure 31 tend to suggest that its absorption rate was comparable to Formula A in the fasted condition, but was increased in the fed state. Therefore, if it can be assumed that the absorption of the preparations was complete by 24 hours, the ke values could be reliably estimated from the three terminal concentration values (at 24, 30 and 36 hours after dosing) by logarithmic transformation of the data and application of linear regression (*Gibaldi and Perrier* 1982a). Compared to the study in section 4, this part of the study has included an additional time point at 30 hours after dosing. Prior to the calculation, the linearity of the terminal slope was inspected by plotting the values on semilog graph paper. If more than three points appeared to be linear, all these would be used for estimation of the ke.

The bioavailability of the two preparations were also compared using the parameters Kr, AUC_o^{12}, AUC_o^{∞}, Tmax, Cmax and lag time. The parameter Kr (absorption rate) was calculated

from the slope of the individual absorption profiles at the point when 50% of the dose had been absorbed. For the other parameters, their numerical values were determined as described previously (section 4.2.4). The Tmax values were corrected for the lag time of absorption. An analysis of variance procedure appropriate for a 2 factorial split-plot (repeated measures in one factor) experimental design (*Kirk* 1968) was used in the statistical analysis of the numerical values of the parameters.

5.2.10 ANALYSIS OF SCINTIGRAPHIC DATA

The images acquired for each volunteer were played back on the computer. Three areas of interest were drawn on the computer screen with a light pen, representing the stomach, large intestine and anatomical marker. This was done for both the anterior and posterior images. The full sequence of images was viewed to check for movement of the volunteer by referring to the anatomical marker. If, on some frames, the subject had moved excessively, these frames were ignored during subsequent analysis. The counts recorded for each area of interest by each camera head were calculated by the computer for each image. These values were corrected for different acquisition time periods and background counts. The background correction was made by subtracting, from each pixel in the area of interest, the mean counts per pixel from a region at the edge of each image. To correct for variation in depth and attenuation of the radioactivity the geometric mean count was calculated from these net counts and corrected for decay (*Tothill et al* 1978). Finally, the corrected geometric mean counts for the stomach and caecum regions were expressed as percentages of the total counts recorded initially when all the administered activity was in the stomach region. The time course of gastric emptying and caecum arrival of pellets in each volunteer could then be estimated from the plot of percentage activity in these two regions versus time.

5.3 RESULTS AND DISCUSSION

5.3.1 COMPARATIVE BIOAVAILABILITY OF FORMULA A AND UNIPHYLLIN

The precision and sensitivity of the HPLC method were not affected by the changes made in the hardware of the analytical system. The coefficients of variations for within day and between day assays, together with the detection limit given in Table 9, are comparable to the values of the previous study (Table 5). In addition, the detector response as well as the standard curve are also linear (Figures 29 and 30).

Serum theophylline concentration data of individual volunteers are tabulated in Appendix A17, whilst the plots of the average serum levels for Formula A and Uniphyllin obtained under the different food status are shown in Figure 31. Since the average determined theophylline content of the Uniphyllin tablets was found to be 286.5 mg, instead of 300 mg as labelled (Table 10), its serum values were normalised for a 300 mg dose. It can be inferred from Figure 31, that there is a marked difference between the serum profiles of Uniphyllin dosed in the fed and fasted state. When Uniphyllin was administered with food, the serum levels can be seen to increase abruptly approximately 2 hours after dosing, and a peak was reached at about 5 hours, reflecting a very rapid rate of absorption occurring during this period. This is clearly illustrated in the absorption profile calculated using the Wagner-Nelson method presented in Figure 32. A major proportion of the administered dose was absorbed during the period between 2 to 5 hours, and absorption was completed within 8 hours. However, during the preceding 2 hours before the start of the rapid absorption phase, the absorption was substantially slower by comparison (Figure 32). It may be related to a delayed gastric emptying induced by food. Unfortunately, this could not be verified in the present study because the gastrointestinal transit behaviour was not amenable to monitoring.

In contrast, the serum profile of Uniphyllin dosed fasted (Figure 31) was characterised by a slower and sustained rate of absorption as seen in its absorption pattern shown in Figure 32. The slope of the absorption profile was generally more gentle in nature, and the absorption process was greatly extended to 18 hours. The slow initial absorption phase observed when the product was dosed with food, was absent from this plot. It is therefore clear from these results, that the absorption of Uniphyllin was modified by food.

Table 9 : PRECISION DATA OF HPLC METHOD
(IN VIVO STUDY II)

Precision of theophylline assay at five levels of concentration

Conc.(ug/ml)	c.v. within day (n=6)	c.v. between day (n=7)
0.5	0.83	6.25
1.0	2.08	3.61
2.0	0.66	3.44
4.0	1.06	1.66
8.0	0.52	1.35

c.v. = coefficient of variation

Detection limit

0.1 ug/ml signal to noise ratio > 12

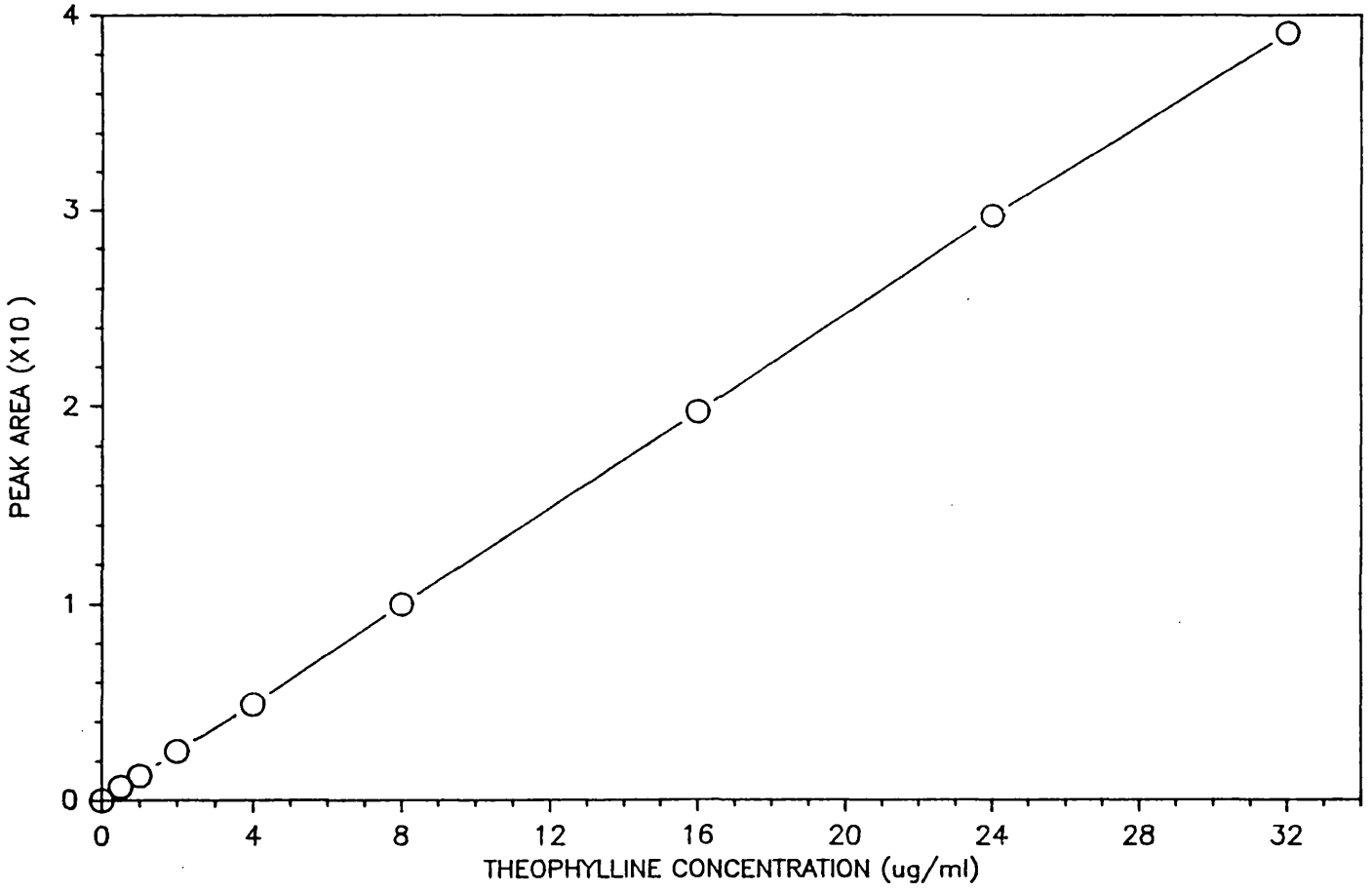


FIGURE 29: PLOT OF THEOPHYLLINE CONCENTRATION VERSUS PEAK AREA SHOWING DETECTOR LINEARITY OF HPLC ANALYSIS (STUDY 2)

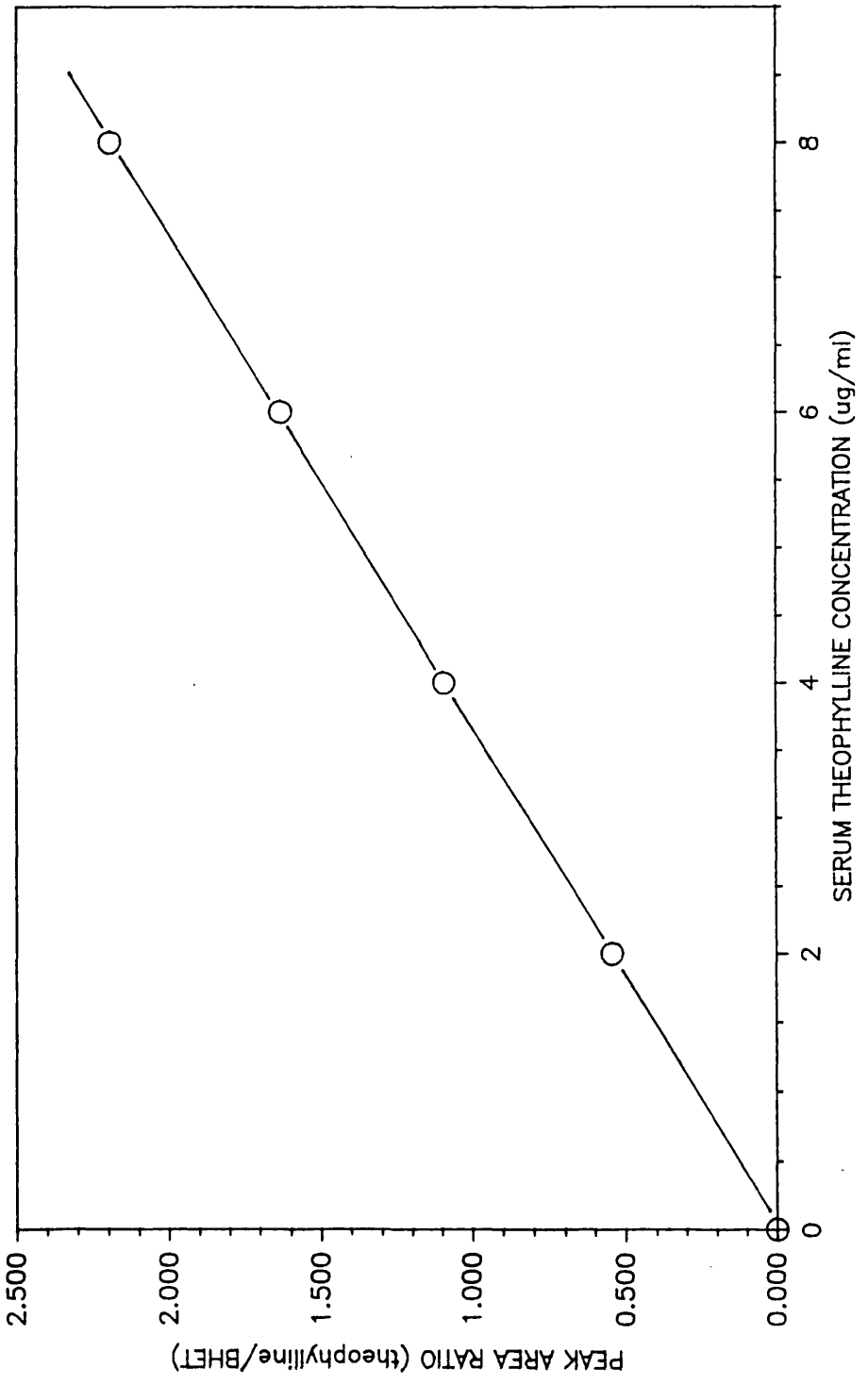


FIGURE 30: STANDARD CURVE OF HPLC ANALYSIS (STUDY 2)

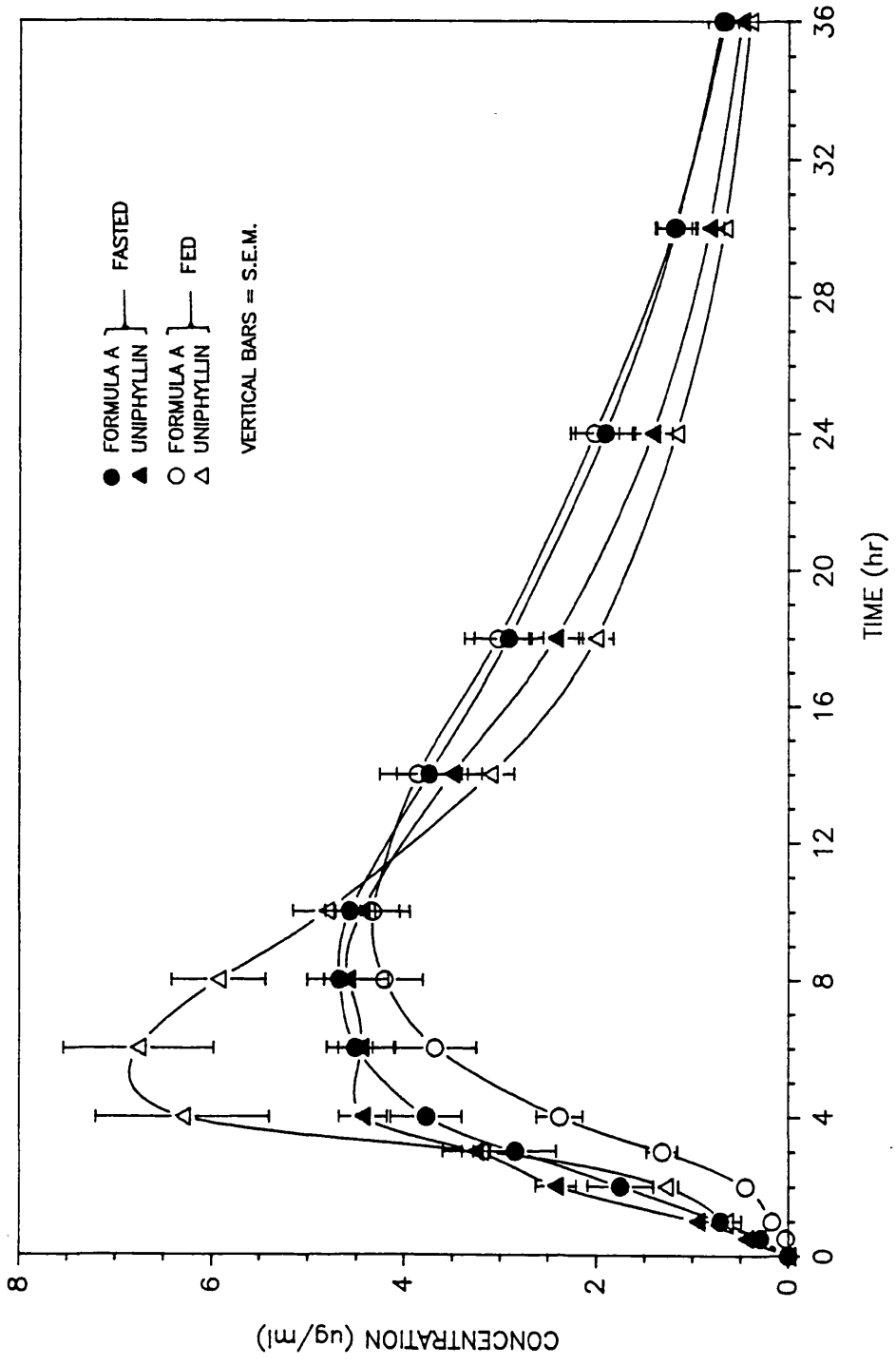


FIGURE 31: AVERAGE SERUM THEOPHYLLINE CONCENTRATION PROFILES OF FORMULA A AND UNIPHYLLIN DOSED FASTED AND FED (DOSE = 300 mg, N = 6)

Table 10 : THEOPHYLLINE CONTENT OF FORMULA A AND UNIPHYLLIN

Preparation	Weight of dosage form(mg)	Content of theophylline(mg)	% by weight
Formula A	400.0	304.1(2.4)	76.1(0.6)
Uniphyllin	401.6(5.6)	286.5(2.3)	71.3(1.2)

Figures in brackets = standard deviation (n=6)

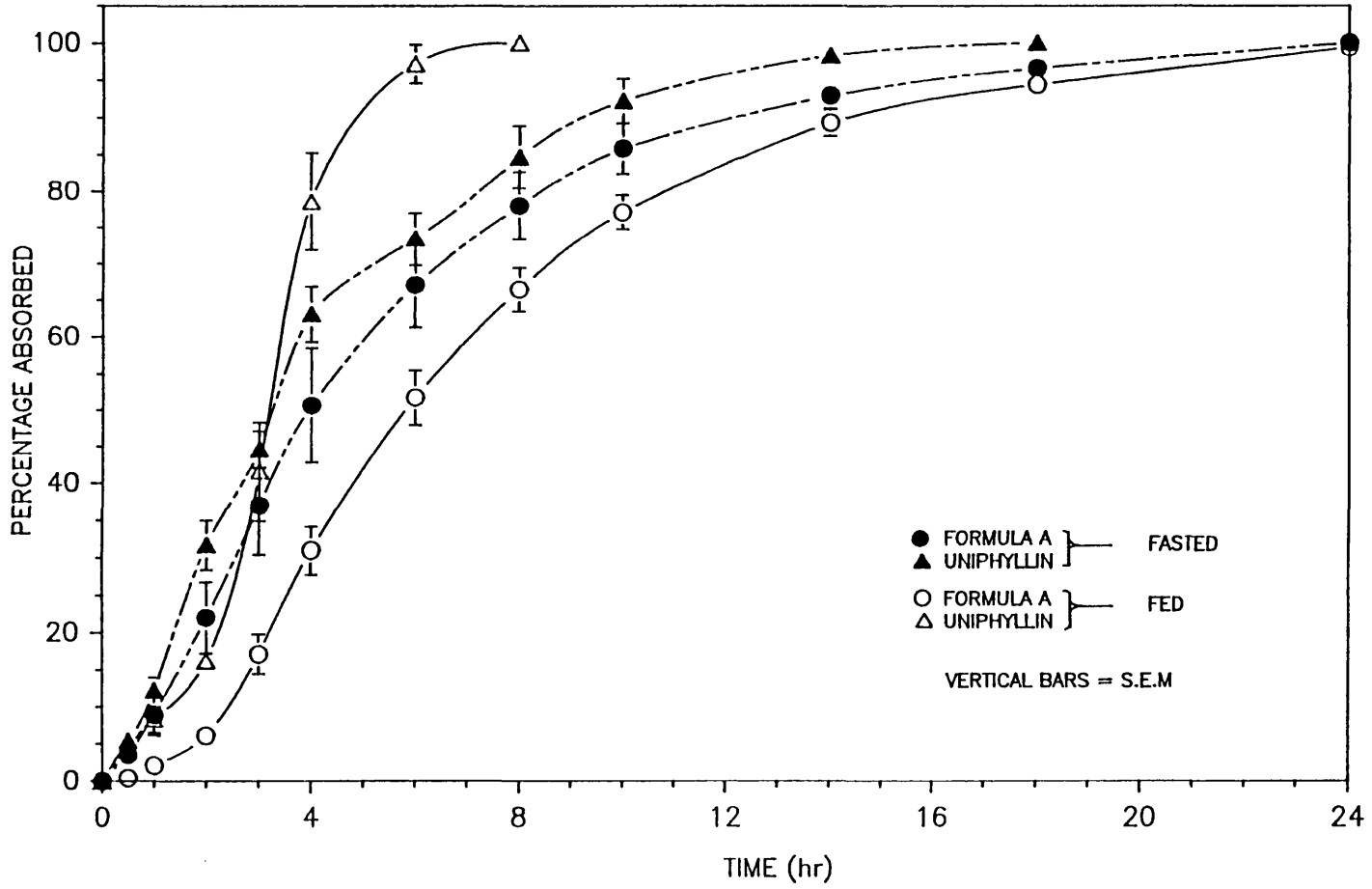


FIGURE 32: PERCENTAGE ABSORBED VERSUS TIME PLOTS OF FORMULA A AND UNIPHYLLIN DOSED FASTED AND FED

In the case of Formula A, both its serum profiles (fed and fasted) appear to be similar, although it may be noted that the serum profile in the fed state was shifted slightly to the right (Figure 31). This was attributed to a delay in the onset of absorption and also the remarkably slow initial absorption phase caused by the presence of food (Figure 32). The latter appears to be a common feature when the preparations were administered in the fed state. The corresponding absorption profiles shown in Figure 32 were essentially parallel, both depicting a relatively slow and sustained rate of absorption. Therefore food did not appear to have any appreciable effect on the rate of absorption with respect to Formula A, other than a delayed and slow initial absorption. It may also be noted that in the fasted state, both the serum and absorption profiles of Uniphyllin were comparable to those of Formula A.

The bioavailability of the two preparations was compared using the relevant parameters, which included the absorption rate (Kr), the maximum serum concentration (Cmax), the time to reach maximum concentration (Tmax), the area under the serum concentration curve from time zero to 12 hours (AUC_o^{12}) and the total area under the serum concentration curve (AUC_o^{∞}). The rate of absorption was compared using the parameters Kr and Tmax, whilst the extent of bioavailability, was compared using AUC_o^{∞}. The parameter, Cmax, which is related to both the rate and extent of bioavailability, provided an additionally useful parameter for comparing the two preparations. On the other hand, AUC_o^{12} permitted the extent of absorption during the initial 12 hours after dosing to be compared.

The numerical values of Kr and Tmax are shown in Tables 11 and 12 respectively, together with a summary of the statistical results. In the analysis of Kr values, food status (factor A) and preparations (factor B) were both found to be statistically significant, and an interaction was present between the two factors. Under the condition where an interaction is statistically significant, it is necessary to analyze the effects of one factor at the various levels of the other and vice versa, whilst the tests of the individual main effects (factor A or B) are of lesser interest. A summary of the results obtained from such an analysis (of the simple main effects) are also given in Table 11. The results indicate that the absorption rates of Formula A and Uniphyllin were statistically different in the fed state, but not when they were dosed fasted. In addition, the absorption rate of Uniphyllin was significantly different with respect to food status, but no statistically significant difference was observed in the case of Formula

Table 11 : NUMERICAL VALUES OF ABSORPTION RATE (kr) OBTAINED WITH FORMULA A AND UNIPHYLLIN DOSED FED AND FASTED, AND ANOVA RESULTS

kr (% hr^{-1})

		Volunteer	Factor B			
			Formula A		Uniphyllin	
Factor A	Fasted	KI	13.7		10.5	
		JE	11.1		22.9	
		SL	16.5	$\bar{x}$ = 12.8	19.6	$\bar{x}$ = 16.7
		NA	20.0	SEM = 2.0	13.8	SEM = 1.8
		VK	7.9		16.0	
		JA	8.1		17.6	
	Fed	LM	12.5		42.2	
		KN	7.7		47.3	
		EY	9.0	$\bar{x}$ = 10.3	33.8	$\bar{x}$ = 39.0
		AM	12.3	SEM = 0.8	29.0	SEM = 3.9
		WI	9.5		29.5	
		WW	10.9		52.2	

ANOVA table

Source	SS	df	MS	F	P
Factor A	583.1	1	583.1	18.83	< 0.002
Error (m. plot)	309.6	10	30.9	-	-
Factor B	1589.2	1	1589.2	40.75	< 0.001
A X B	923.8	1	923.8	23.68	< 0.001
Error (s. plot)	390.0	10	39.0	-	-
Total	3795.7	23	-	-	-

ANOVA table (simple main effects)

Source	SS	df	MS	F	P
Between A at b1	19.5	1	19.5	0.56	(NS)
Between A at b2	1487.4	1	1487.4	42.50	< 0.001
Error (w. cell)	699.6	20	35.0	-	-
Between B at a1	44.8	1	44.8	1.15	0.309(NS)
Between B at a2	2468.2	1	2468.2	63.29	< 0.001
Error (s. plot)	390.0	10	39.0	-	-

Table 12 : NUMERICAL VALUES OF T_{max} OBTAINED WITH FORMULA A AND UNIPHYLLIN DOSED FED AND FASTED, AND ANOVA RESULTS

T_{max} (hr)

		Volunteer	Factor B			
			Formula A		Uniphyllin	
Factor A	Fasted	KI	7.8		10.0	
		JE	9.2		8.0	
		SL	5.8	$\bar{x}$ = 8.1	4.0	$\bar{x}$ = 7.3
		NA	6.0	SEM = 0.7	4.0	SEM = 1.1
		VK	10.0		10.0	
		JA	9.5		8.0	
	Fed	LM	9.6		6.0	
		KN	8.2		4.0	
		EY	9.5	$\bar{x}$ = 8.9	6.0	$\bar{x}$ = 6.0
		AM	7.6	SEM = 0.4	8.0	SEM = 0.7
		WI	9.7		8.0	
		WW	8.7		4.0	

ANOVA table

Source	SS	df	MS	F	P
Factor A	0.38	1	0.38	0.07	(NS)
Error (m. plot)	58.22	10	5.82	-	-
Factor B	19.44	1	19.44	12.62	0.005
A X B	7.04	1	7.04	4.57	0.058
Error (s. plot)	15.38	10	1.54	-	-
Total	100.46	23	-	-	-

ANOVA table (simple main effects)

Source	SS	df	MS	F	P
Between A at b1	2.09	1	2.09	0.57	(NS)
Between A at b2	5.33	1	5.33	1.45	0.242(NS)
Error (w. cell)	73.60	20	3.68	-	-
Between B at a1	1.54	1	1.54	1.00	0.341(NS)
Between B at a2	24.94	1	24.94	16.19	< 0.003
Error (s. plot)	15.38	10	1.54	-	-

A. Therefore the statistical analyses of the differences in Kr are in accord with the qualitative differences observed in the serum and absorption profiles shown in Figures 31 and 32 respectively.

In the analysis using Tmax values, only factor B was statistically significant (Table 12) at the significance level of P = 0.05. However, because the interaction between the factors was of particular interest, a significance level of up to P = 0.1 was deemed acceptable. Since the interaction term was significant at this level, the simple main effects were further analyzed and a summary of the results is shown in Table 12. As with the parameter Kr, a statistical difference between corresponding values of Tmax was observed between Uniphyllin and Formula A in the fed mode, but not in the fasted condition. Also, no statistical difference was observed in values of Tmax for Formula A with respect to food status. However, whilst the analysis with Kr indicated a significant difference in the absorption rate of Uniphyllin dosed under the different food status, this was not observed with the parameter Tmax, in conflict with the observations made in the serum concentration and absorption profiles depicted in Figures 31 and 32. This discrepancy illustrates the limitation of the directly observed Tmax values (which afterall is a single point determination) for comparing the absorption rate in the present analysis. As emphasized previously, the rapid absorption phase of Uniphyllin dosed with food (and where the bulk of the dose was absorbed) occurred at approximately 2 hours after dosing, and was preceded by an initial slow absorption phase. Therefore, the Tmax values would be unnecessarily large and not truly reflect the absorption rate. The same situation would arise if the times for 50% of the drug to be absorbed (t50%) were used.

The numerical values of Cmax and AUC_o^{12}, together with a summary of the statistical results are given in Tables 13 and 14 respectively. Consistent results were obtained in the statistical analyses using the values of these two parameters. A significant interaction between factors A and B was present. Further analysis of the simple main effects yielded results that were also in accord with those obtained with the parameter Kr. A statistical difference was observed between Uniphyllin and Formula A in the fed mode, but not in fasted condition. On the other hand, the fed and fasted values of these parameters were statistically different for Uniphyllin only, and not with Formula A.

Table 13 : NUMERICAL VALUES OF C_{max} OBTAINED WITH FORMULA A AND UNIPHYLLIN DOSED FED AND FASTED, AND ANOVA RESULTS

C_{max} (ug ml^{-1})

		FACTOR B					
		Volunteer	Formula A		Uniphyllin		
FACTOR A	fasted	KI	4.80		4.15		
		JE	5.44		5.71		
		SL	4.26	$\bar{x}$ = 4.89	4.25	$\bar{x}$ = 4.95	
		NA	5.18	SEM = 0.19	4.79	SEM = 0.28	
		VK	4.46		5.00		
		JA	5.18		5.77		
	fed	LM	6.15		9.33		
		KN	4.27		9.71		
		EY	4.26	$\bar{x}$ = 4.34	7.38	$\bar{x}$ = 7.16	
		AM	3.49	SEM = 0.38	4.91	SEM = 0.84	
		WI	3.81		4.93		
		WW	4.07		6.70		

ANOVA table

Source	SS	df	MS	F	P
Factor A	4.18	1	4.18	1.84	0.202(NS)
Error (m. plot)	22.72	10	2.27	-	-
factor B	12.41	1	12.41	18.75	0.001
A X B	11.43	1	11.43	17.26	0.002
Error (s. plot)	6.62	10	0.66	-	-
Total	57.36	23	-	-	-

ANOVA table (simple main effects)

Source	SS	df	MS	F	P
Between A at b1	0.89	1	0.89	0.61	(NS)
Between A at b2	14.72	1	14.72	10.03	0.005
Error (w. cell)	29.34	20	1.47	-	-
Between B at a1	0.01	1	0.01	0.02	(NS)
Between B at a2	23.82	1	23.82	36.04	<0.001
Error (s. plot)	6.62	10	0.66	-	-

Table 14 : NUMERICAL VALUES OF AUC_O^{12} OBTAINED WITH FORMULA A AND UNIPHYLLIN DOSED FED AND FASTED, AND ANOVA RESULTS

AUC_O^{12} (ug ml^{-1} hr)

		FACTOR B				
		Volunteer	Formula A		Uniphyllin	
FACTOR A	fasted	KI	44.7		40.4	
		JE	39.6		50.3	
		SL	39.4	$\bar{x}$ = 42.61	34.4	$\bar{x}$ = 44.18
		NA	48.1	SEM = 1.51	43.9	SEM = 2.69
		VK	39.3		43.7	
		JA	44.5		52.4	
	fed	LM	49.6		68.9	
		KN	31.4		63.8	
		EY	31.9	$\bar{x}$ = 34.01	53.5	$\bar{x}$ = 53.45
		AM	29.8	SEM = 3.21	43.8	SEM = 4.49
		WI	27.9		41.0	
		WW	33.4		49.8	

ANOVA Table

Source	SS	df	MS	F	P
Factor A	0.7	1	0.7	0.007	(NS)
Error (m. plot)	953.0	10	95.3	-	-
Factor B	662.6	1	662.6	26.6	<0.001
A X B	479.0	1	479.0	19.24	0.001
Error (s. plot)	248.9	10	24.9	-	-
Total	2344.2	23	-	-	-

ANOVA Table (simple main effects)

Source	SS	df	MS	F	P
Between A at b1	221.7	1	221.7	3.69	0.07(NS)
Between A at b2	258.0	1	258.0	4.29	0.05
Error (w. cell)	1201.9	20	60.1	-	-
Between B at a1	7.3	1	7.3	0.29	(NS)
Between B at a2	1134.3	1	1134.3	45.55	<0.001
Error (s. plot)	248.9	10	24.9	-	

Table 15 contains the numerical values of AUC_0^{∞} and a summary of the statistical results. Factors A and B, and AB interaction were not statistically significant. Therefore, it can be concluded that the extent of bioavailability within each preparation and between the preparations was not statistically different under both food conditions. A further comparison was made between the fed and fasted values of lag time recorded for Formula A (Table 16) using an independent t-test. No statistical difference was observed between the fed and fasted values. In the case of Uniphyllin, no lag time was recorded in both food conditions.

On the basis of the analyses above, it can therefore be concluded that Formula A and Uniphyllin were bioequivalent when dosed in the fasted mode. However, in the fed condition, the absorption rate of Uniphyllin was significantly increased, whereas that of Formula A was not. In comparison, the extent of absorption of each preparation and between the preparations was not significantly different under both food conditions.

It is generally recognised that, in the split-plot experimental design, the test on factor B and AB interactions (within subject effects) has greater power than the test on factor A (between subject effects). This results from the fact that variation within a subject is usually smaller than variation among subjects (*Kirk* 1968). In the present study, the effects of food status were judged as more readily discernable than the effects of the preparations. As such, they were assigned as factor A and B respectively in the study. Undeniably, a complete 4-way crossover of the drug treatments would have been a better study design, that is, each subject would receive both preparations under both food status. However, due to the high possibility of volunteers dropping out during the course of such a long study, and also the difficulties in recruiting volunteers who would consent to four drug treatments, the split-plot design was a satisfactory compromise. Individual exposure to the radiation dose was also reduced. Moreover, the test for the AB interaction, which was of interest in the study, has the same power as the test for factor B. The assumptions involved in the ANOVA test discussed in section 4.3, apply in the present analysis. All the sample variances were found to comply when evaluated for homogeneity.

The *in-vitro* release profiles of the two preparations as a function of pH are shown in Figure 33. The rate of release of Uniphyllin was generally slower than that of Formula A. Whilst the dissolution of the latter was essentially unaffected by pH, the dissolution of Uniphyllin

Table 15 : NUMERICAL VALUES OF AUC_o^α OBTAINED WITH FORMULA A AND UNIPHYLLIN DOSED FED AND FASTED, AND ANOVA RESULTS

AUC_o^α (ug ml^{-1} hr)

		FACTOR B				
		Volunteer	Formula A		Uniphyllin	
FACTOR A	fasted	KI	106.7		85.6	
		JE	91.4		91.2	
		SL	74.6	$\bar{x}$ = 101.5	56.2	x = 91.41
		NA	85.1	SEM = 8.7	93.2	SEM = 8.3
		VK	128.2		111.8	
		JA	123.0		110.3	
	fed	LM	145.5		120.6	
		KN	77.4		103.8	
		EY	92.3	$\bar{x}$ = 93.3	92.8	$\bar{x}$ = 93.4
		AM	78.4	SEM =10.6	89.5	SEM = 6.9
		WI	81.8		75.2	
		WW	84.4		78.7	

ANOVA table

Source	SS	df	MS	F	P
Factor A	56.83	1	56.83	0.07	(NS)
Error (m.plot)	8077.44	10	807.74	-	-
Factor B	149.94	1	149.94	1.37	0.268(NS)
A X B	157.34	1	157.34	1.44	0.257(NS)
Error (s. plot)	1093.92	10	109.39	-	-
Total	9535.47	23	-	-	-

Table 16 : NUMERICAL VALUES OF LAG TIME OBTAINED WITH FORMULA A DOSED FED AND FASTED

Volunteer		Lag-time(hr)	
Fasted	Fed	Fasted	Fed
KI	LM	0.2	0.4
JE	KN	0.8	1.8
SL	EY	0.2	0.5
NA	AM	0.0	0.4
VK	WI	0.0	0.3
JA	WW	0.5	0.3
		* P = 0.246 (NS)	

* independent t-test

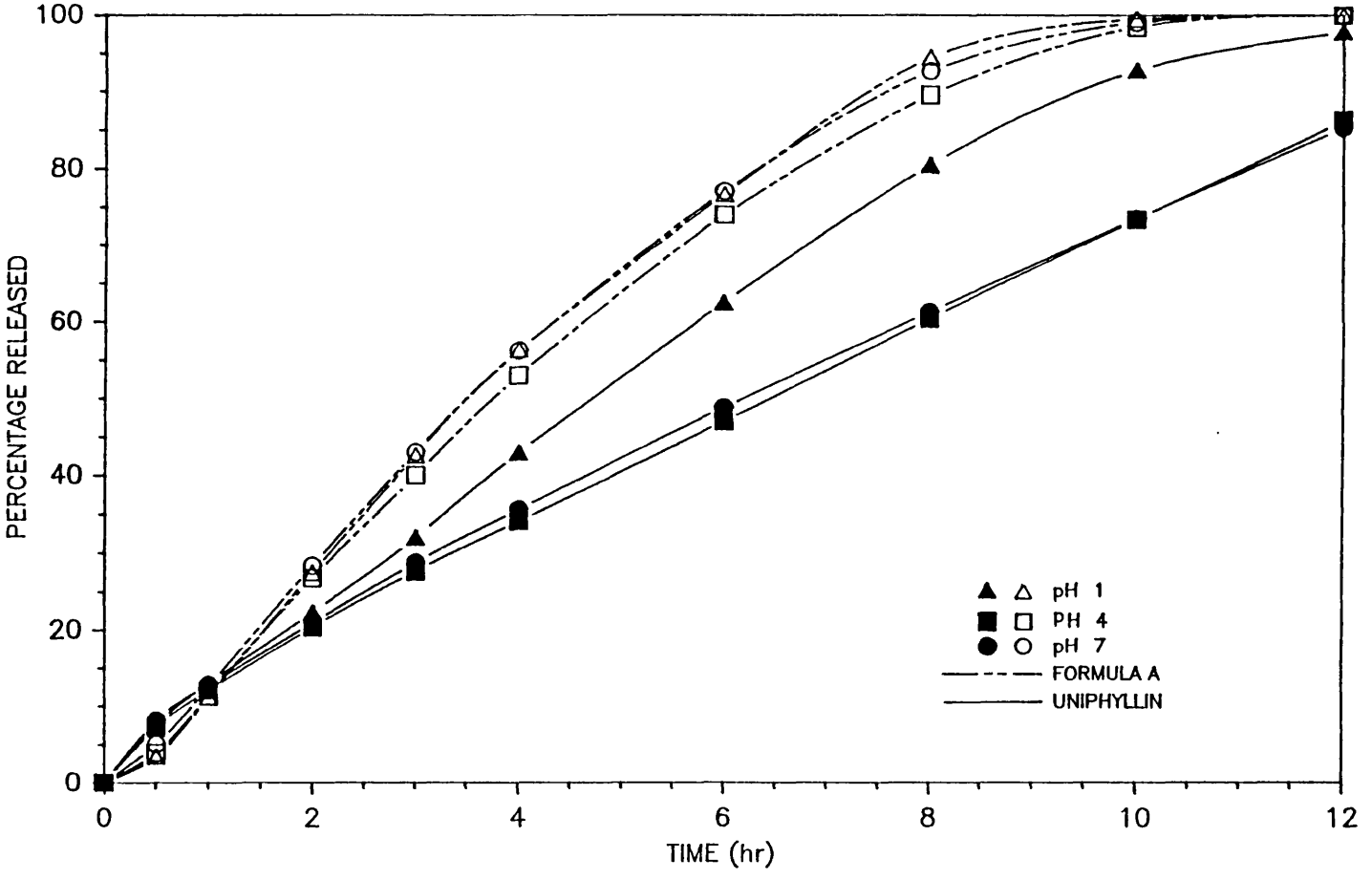


FIGURE 33: IN–VITRO THEOPHYLLINE RELEASE FROM UNIPHYLLIN TABLETS AND FORMULA A AS A FUNCTION OF pH (N = 6)

was observed to be faster at pH 1, but remained similar in profile at pH 4 and 7. *Po et al* (1990) reported similar findings with Uniphyllin. It should be noted however, that the initial rate of dissolution of Uniphyllin was pH independent, and a difference in release rate only became apparent after 2 hours of dissolution. Therefore, any attempt to alter the pH of the dissolution medium in a stepwise manner (to mimic the pH changes of the gut) will result in a dissolution profile that is basically similar to those obtained at the two higher pH values. Hence, the plots shown in Figures 34 and 35, interrelating the *in-vivo* and *in-vitro* data, were constructed using the dissolution profiles determined at pH 7. An intensity factor (described in section 4.3) of 1.4 was obtained for Formula A and used in constructing all the plots in Figure 34. The *in-vivo* absorption of Formula A appears to be slower than the *in-vitro* release, as indicated by the value of the intensity factor, c.f. previous study. In comparison, the value obtained with Uniphyllin was less than unity, suggesting that the *in-vitro* release was slower than the *in-vivo* absorption.

In Figures 34 and 35, the plots for Formula A show a relatively linear relationship between the *in-vivo* and *in-vitro* results. By and large, they are similar to the plots obtained in the previous study (Figures 27 and 28). The non coincident but parallel nature of the two plots (fed and fasted) depicts a constant delay in absorption times attributable to food. The correlation coefficients of the plots in Figure 34 are 0.9957, $P < 0.001$ (fed) and 0.9895, $P < 0.001$ (fasted) and in Figure 35 are 0.9816, $P < 0.001$ (fed) and 0.9785, $P < 0.001$ (fasted). In contrast, the *in-vivo/in-vitro* relationship obtained with Uniphyllin was more complex. Both the plots for the fed and fasted states shown in Figure 34 are essentially sigmoidal in nature with correlations coefficients (R) of 0.9615 ($P < 0.001$) and 0.9445 ($P < 0.001$) respectively. Although a fairly linear relationship is depicted by the plots in Figure 35, the plot for the fed state has an almost zero gradient, and in both cases the *in-vivo* absorption times were greatly overestimated *in-vitro*. The correlation coefficients for the two plots are 0.9258 ($P < 0.001$) for the fed state and 0.9756 ($P < 0.001$) for the fasted state.

Notwithstanding that a less satisfactory *in-vivo/in-vitro* relationship was obtained with Uniphyllin, in particular the sigmoidal nature of the plots shown in Figure 34, a highly statistically significant correlation was still indicated. This illustrates the deficiency of the correlation coefficient for interrelating the data. Moreover, the R value is dependent on the range covered by the data. An increase in the range covered will lead to a corresponding

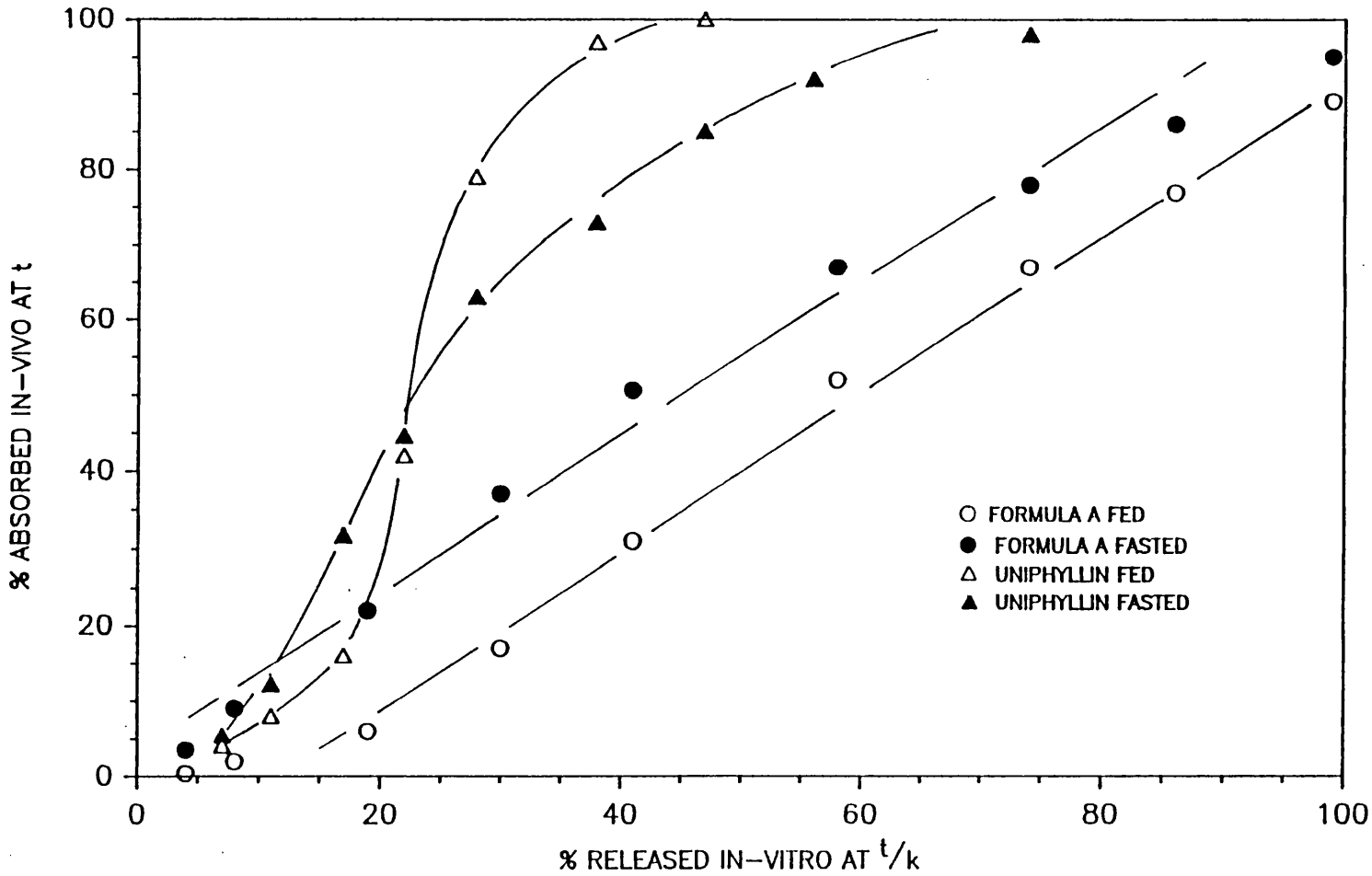


FIGURE 34: PLOT OF PERCENTAGE ABSORBED IN-VIVO AT TIME t VERSUS PERCENTAGE RELEASED IN-VITRO AT t/k FOR FORMULA A AND UNIPHYLLIN DOSED FASTED AND FED

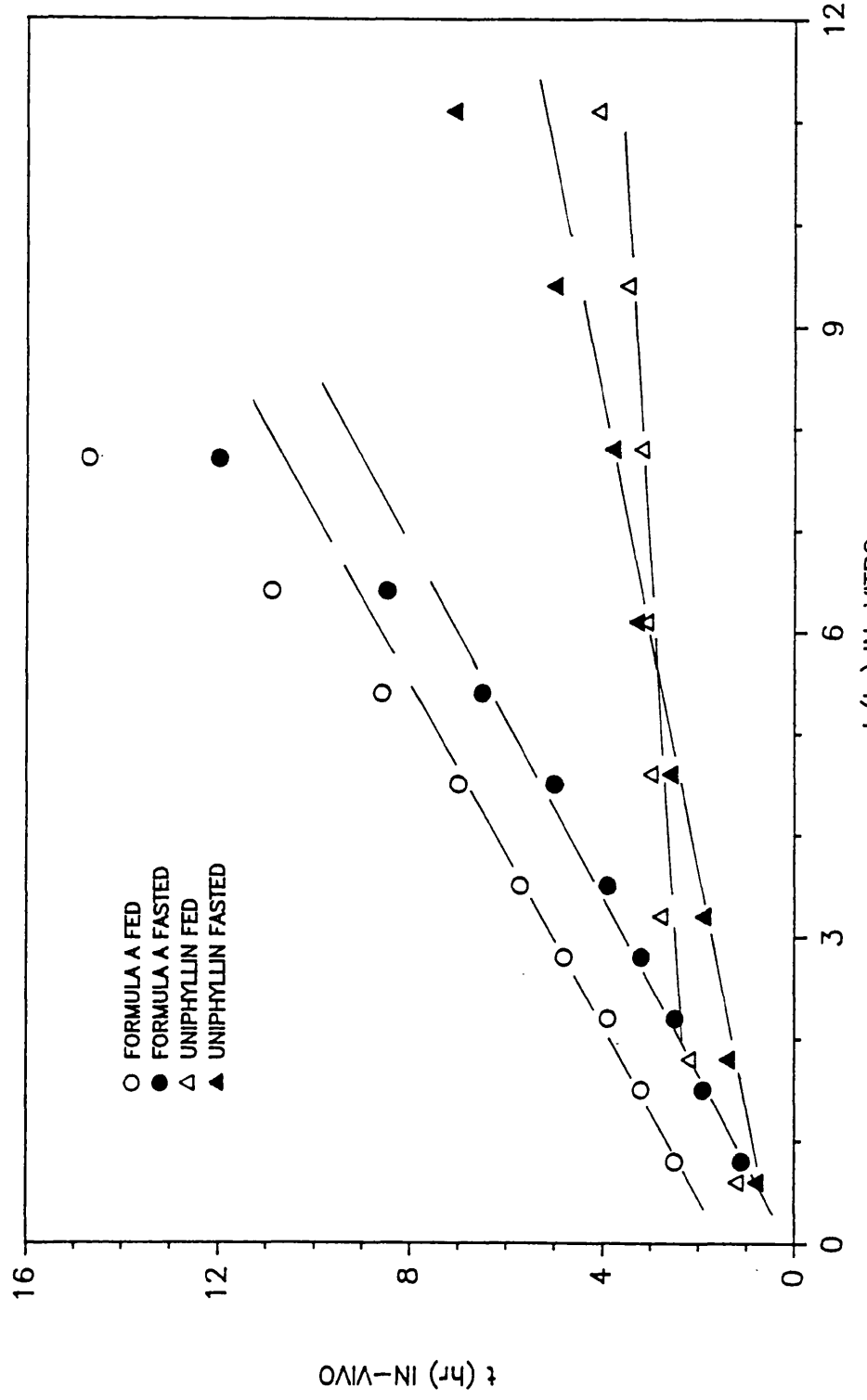


FIGURE 35: PLOT OF TIME OF ABSORPTION IN-VIVO VERSUS TIME FOR EQUAL PERCENTAGES RELEASED IN-VITRO FOR FORMULA A AND UNIPHYLLIN DOSED FASTED AND FED

increase in R value (*Westgard and Hunt* 1973). Also, since the R value is a measure of a linear relationship between two variables (*Snedecor and Cochran* 1980; *Milton and Tsokos* 1983), its application in situations where the relationship is clearly non-linear may be inappropriate (*Duncan et al* 1983). In view of all these, a visual and critical examination of the plots (as shown in Figures 34 and 35) will shed more light on the relationship.

The results of the present study indicate that food modified the absorption of Uniphyllin, which has a pH dependent *in-vitro* release, but was without influence with Formula A where the release was pH independent. Although similar findings have been reported by Delhotal-Landes *et al* (1988), no generalisation can be made regarding the association between pH dependency of *in-vitro* release and food induced modification of *in-vivo* absorption (*Karim* 1988). This is because food does not only cause changes in the gastric pH, but may also modulate other physiological functions of the gut such as gastric emptying and secretions of enzymes and hormones (*Karim* 1988). Additionally, food can also have a direct interaction with the dosage forms. These factors may singly or in combination contribute to changes in the *in-vivo* release/absorption. Studies in which only the *in-vivo* pH was modified, have achieved more promising results with respect to the association between *in-vitro* pH dependent release and the *in-vivo* absorption characteristics (*Myhre and Walstad* 1983; *Vashi and Meyer* 1988).

Whilst, the absorption rate of Formula A was unaffected by food, a delay in absorption was noted. This feature was also observed in many of the products reviewed in section 1.3.2, and may be ascribed to a delayed gastric emptying. On the other hand, the dramatic increase in the absorption rate of Uniphyllin dosed in the fed state, could be attributed to degradation of its controlled release mechanism brought about by the digestive activity of the stomach in the presence of food (*Lagas and Jonkman* 1983). In recent years, interests have been shown in designing *in-vitro* test systems that can predict food effects, and the initial findings appear encouraging (*Wearley et al* 1985; *Maturu et al* 1985, *El-Arini et al* 1990). Such tests can be a useful tool in studying factors which may be responsible for dissolution-related food effects on the absorption of sustained release products.

5.3.2 GASTROINTESTINAL TRANSIT STUDIES

The densities of the coated theophylline and Amberlite pellets are given in Table 17. Consistent measurements were obtained for each type of pellets, and the standard deviation was effectively zero. Although Devereux (1987) and Clarke (1989) observed a slight increase in density in some batches of products after radiolabelling, this was not observed with the Amberlite pellets and the density remained unchanged. Before labelling, the internal pores of the pellets were sealed by the film coat. Therefore, it was suggested that dissolution of the soluble component in the coat during the labelling process caused the internal pores to be exposed, resulting in a smaller effective volume being measured by the air comparison pycnometer. As a consequence, a higher density was recorded. Both the theophylline and Amberlite pellets have essentially similar density. Therefore, the latter is suitable for use as the radioactive marker in monitoring the theophylline pellets during the *in-vivo* study. Because the two types of pellets have similar size range and density, it was assumed that they also have the same gastrointestinal transit properties.

The radiolabelling process is based on an exchange of pertechnetate (TcO_4^-) anion with the chloride anion of the ion exchange resin. The radiolabel uptake efficiency of the Amberlite pellets with respect to the amount of activity added was 43% (Table 18). The variability in uptake between batches was acceptable (coefficient of variation = 6.5%). By comparison, the percentage uptake was higher than that reported by Devereux (1987) and Clarke (1989), although the latter also reported comparable uptake efficiency with some batches of pellets. The differences in uptake of the radiolabel could be attributed to variations in porosity of the film coat (*Clarke* 1989).

Results obtained from *in-vitro* testing of the stability of 99m-Tc binding to the pellets are given in Table 19. In all the three media used, less than 4.0% of the radiolabel was released, being lower than the values reported by Devereux (1987) and Christensen *et al* (1983) with broadly similar formulations. Therefore, the radiolabelled pellets were considered satisfactory for *in-vivo* administration. Release of the radiolabel in the gastrointestinal tract is undesirable and should be kept to a minimum, because it has the potential of being systemically absorbed and localised in other organs. Furthermore, it may interfere with the clarity of images taken. All the pellets were found to remain intact after the experiments.

Table 17 : DENSITY OF FORMULA A AND 5% AMBERLITE PELLETS

	Density(g cm^{-3})
Formula A	1.45(<0.01)
Amberlite pellets	1.48(<0.01)
Labelled Amberlite pellets	1.46(<0.01)

Figures in brackets = standard deviation (n=4)

Table 18 : UPTAKE OF 99m-Tc BY 5% AMBERLITE PELLETS

Mean % uptake	Standard deviation	Number of determinations
43.2	2.8	12

Table 19 : IN-VIVO RELEASE OF 99m-Tc FROM 5% AMBERLITE PELLETS AT DIFFERENT VALUES OF PH

	Simulated gastric juice pH=1.2	Phosphate buffer pH=4	Simulated intestinal juice pH=7.5
Time of testing (hr)	4.0	14.0	14.0
% released	2.1(1.1)	2.5(1.5)	3.7(1.5)

Figures in brackets = standard deviation (n=6)

Table 20 : DISTRIBUTION OF RADIOACTIVITY AMONG LABELLED PELLETS IN TWO BATCHES PREPARED AS FOR ADMINISTRATION

Mean sample deviation (%)	1) 2.8 2) 3.8
Standard deviation	1) 2.0 2) 2.2

Since the distribution of the dosage form within the various regions of the gastrointestinal tract was quantitated using the radioactive measurements (section 5.2.10), it is essential that the radioactivity was uniformly distributed among the labelled pellets. The mean sample deviations with respect to the distribution of the activity among the pellets (irrespective of sign) are shown in Table 20. It can be inferred that the distribution was sufficiently uniform, and acceptable for the *in-vivo* study.

(a) gastric emptying

The curves depicting the gastric emptying and caecum arrival of pellets for the individual volunteers are shown in Figures 36 to 47. Wide intersubject variations were observed with respect to both the gastric emptying and caecum arrival curves. However, on closer examination of the figures, it is revealed that a majority of the volunteers dosed in the fed state, exhibited a delayed gastric emptying and caecum arrival, with an apparent shift of the curves to the right. A clearer illustration is shown in Figure 48 when all the curves were plotted on the same graph.

The gastric emptying of a single unit non-disintegrating dosage form is a simple all or none process, whilst the emptying of multiunit formulations is more complex and variable. Hunter *et al* (1983) observed at least 5 different patterns of emptying of encapsulated formulations. Therefore, it is not possible to describe the emptying process in a concise manner by fitting the data points to a single mathematical model (*Clarke* 1989). In the case of liquid or solid meals, emptying usually occurs in a constant pattern. For example, liquid emptying is generally accepted as being a monoexponential process (*Fisher et al* 1982; *Harris et al* 1987; *Heading et al* 1976). On the other hand, it has been suggested that digestible solids empty in a linear fashion with time (*Harris et al* 1987; *Heading et al* 1976) subsequent to a lag phase, during which the solid particles are reduced to a size small enough to be emptied. In view of the constancy of emptying patterns exhibited by liquid and solid meals, mathematical fitting of the data is more applicable and has been applied by Cook *et al* (1975), Elashoff *et al* (1982, 1983) and Kim *et al* (1981).

Frequently, the gastric emptying (or caecum arrival) curves of more than one subject have been presented as a single average curve (*Christensen et al* 1985; *Davis et al* 1984, 1987; *O'Reilly et al* 1987; *Ollerenshaw et al* 1987; *Urbain et al* 1989; *Sugito et al* 1990), and linear

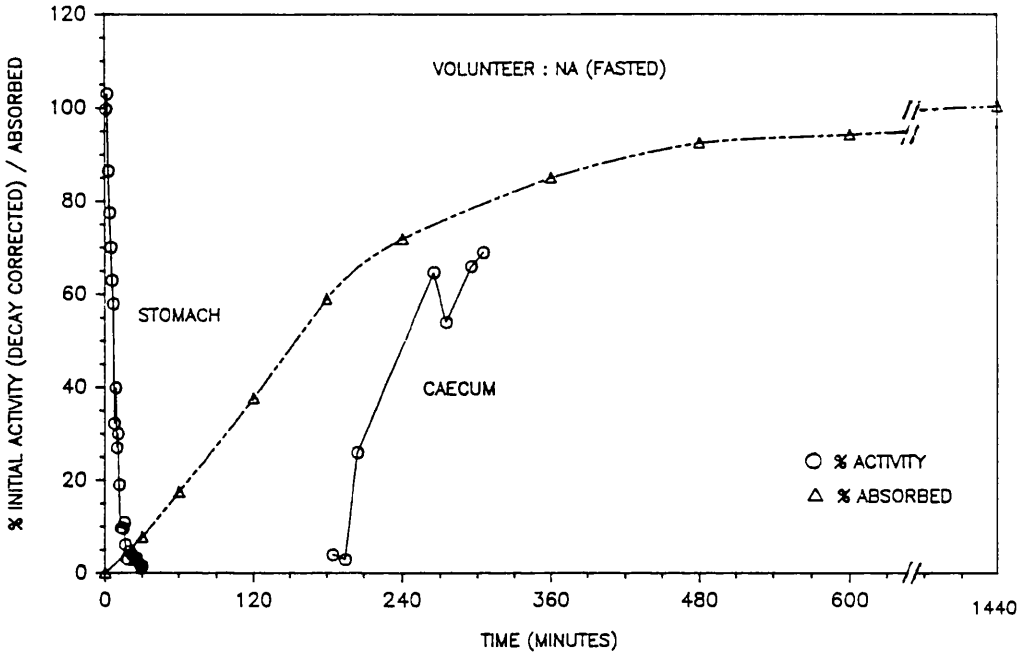


FIGURE 36: GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER NA (FASTED)

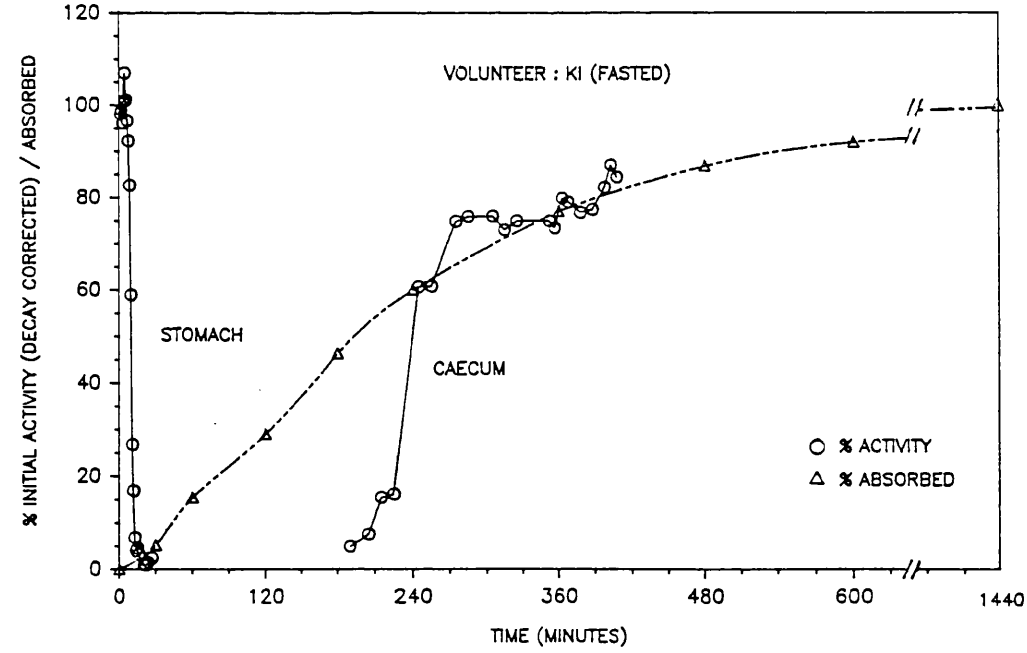


FIGURE 37: GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER KI (FASTED)

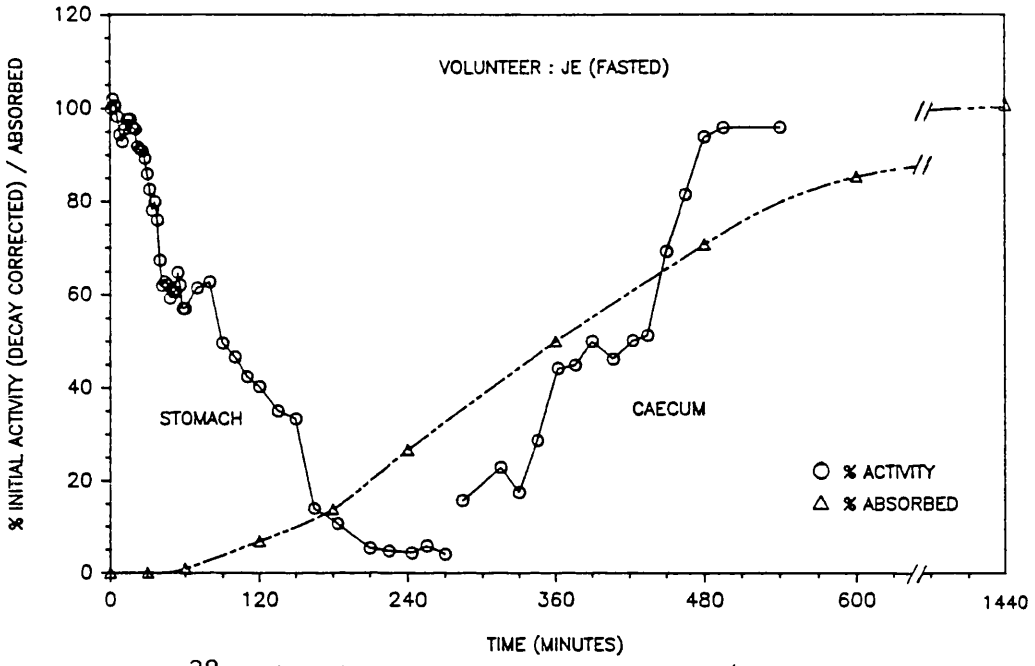


FIGURE 38 GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER JE (FASTED)

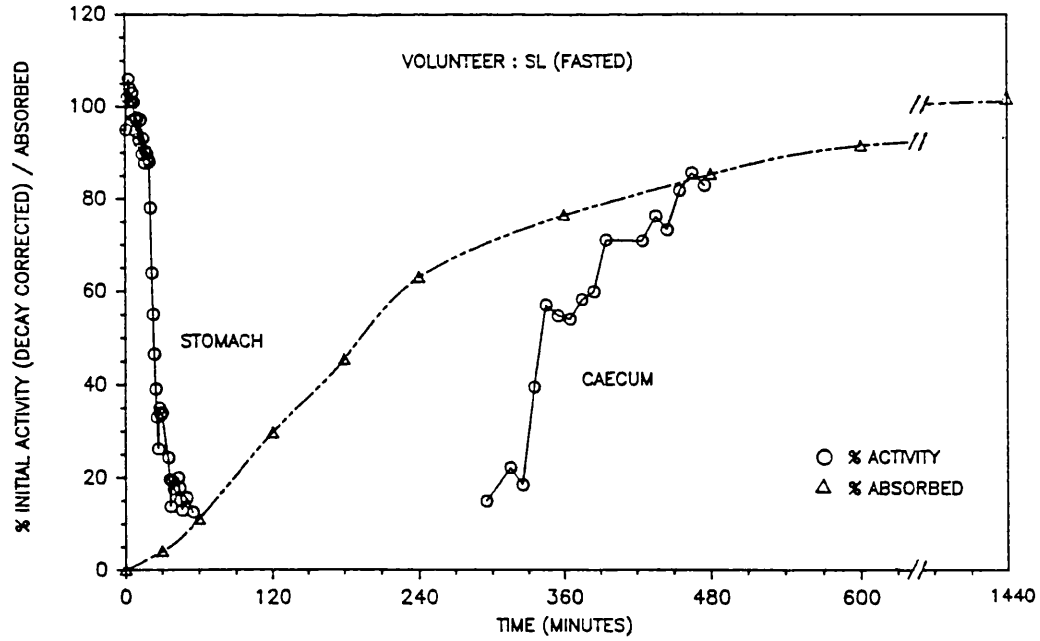


FIGURE 39 GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER SL (FASTED)

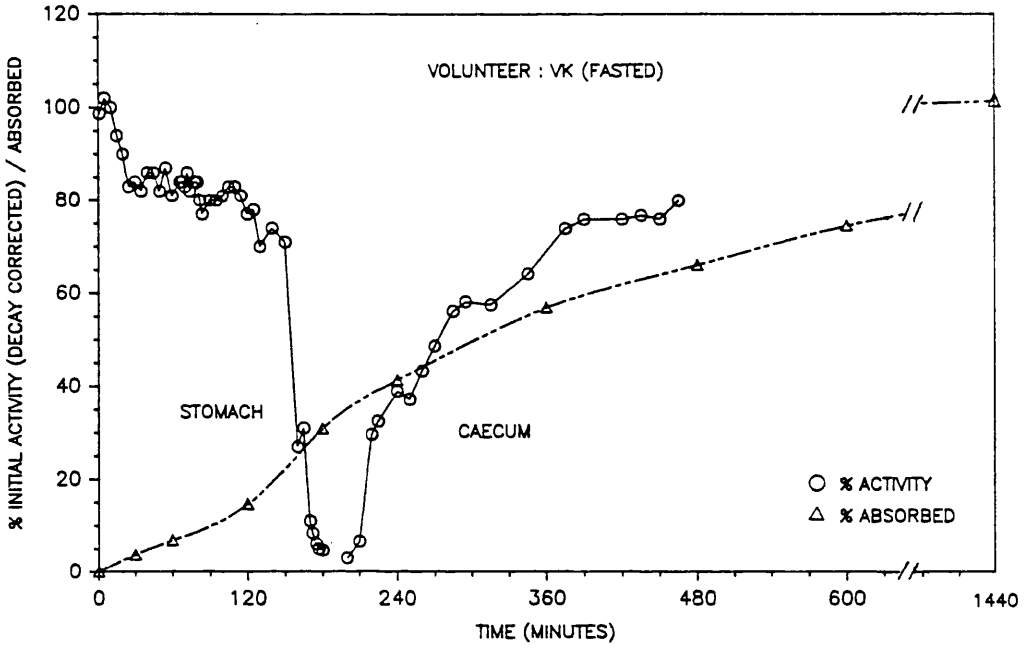


FIGURE 40 : GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER VK (FASTED)

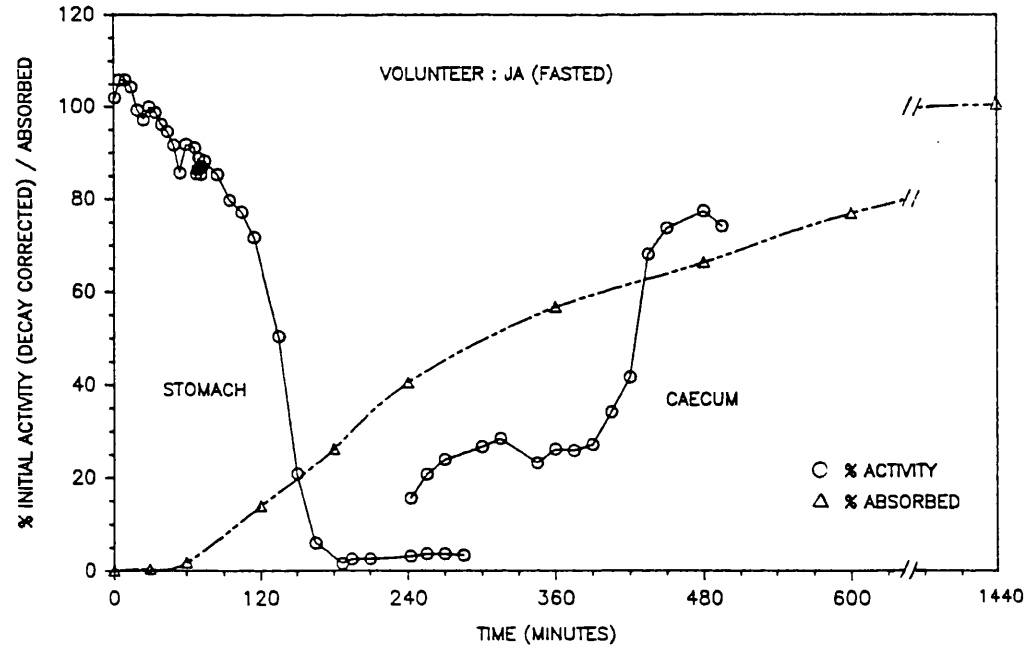


FIGURE 41 : GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER JA (FASTED)

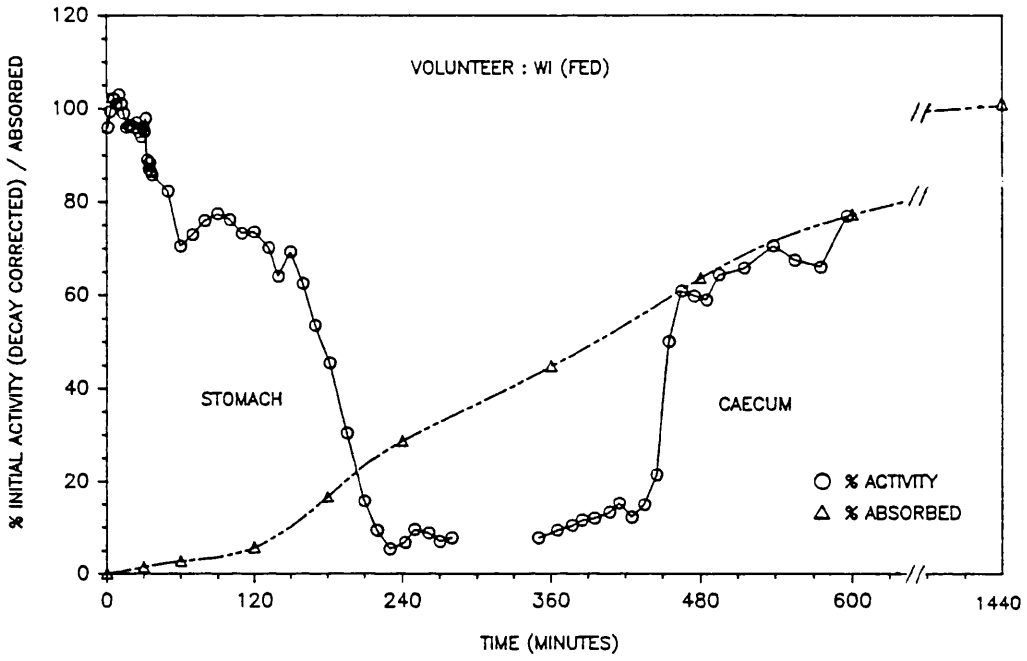


FIGURE 42 : GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER WI (FED)

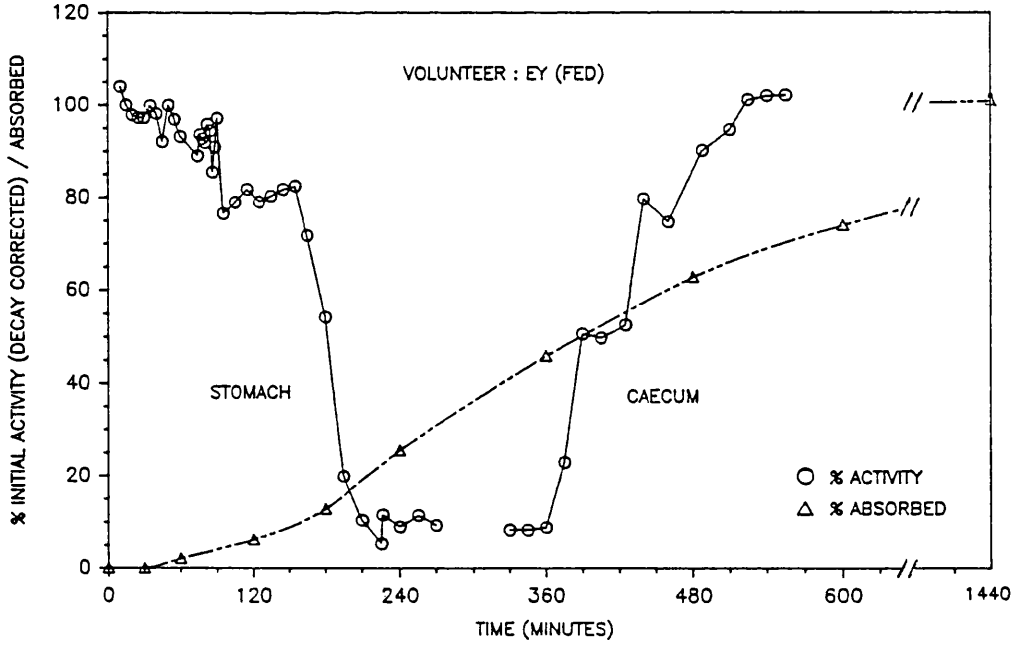


FIGURE 43 : GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER EY (FED)

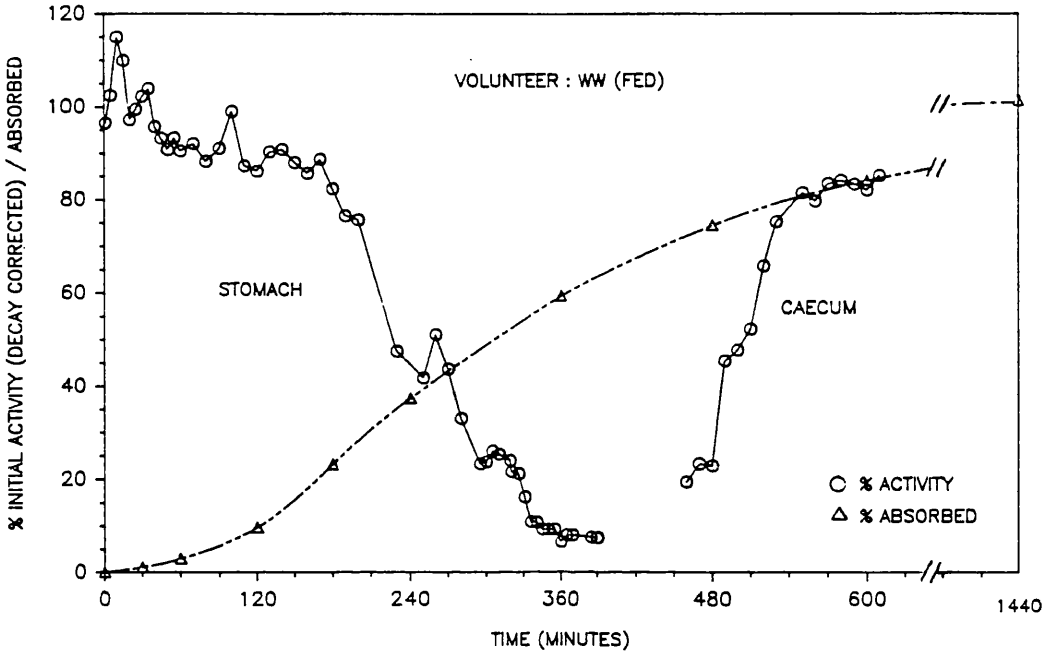


FIGURE 44 :GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER WW (FED)

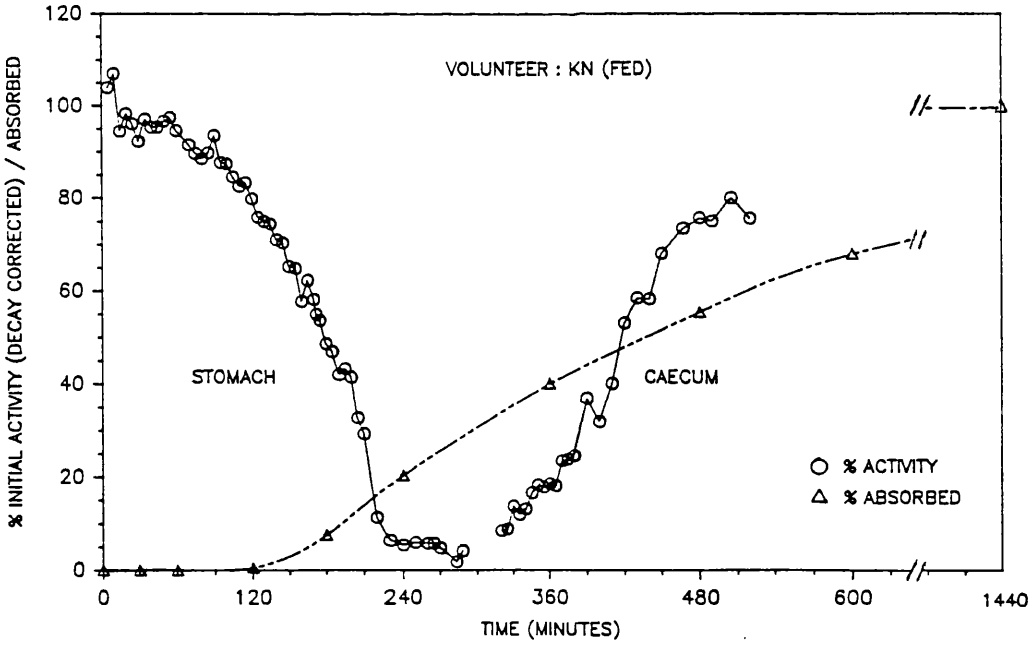


FIGURE 45 :GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER KN (FED)

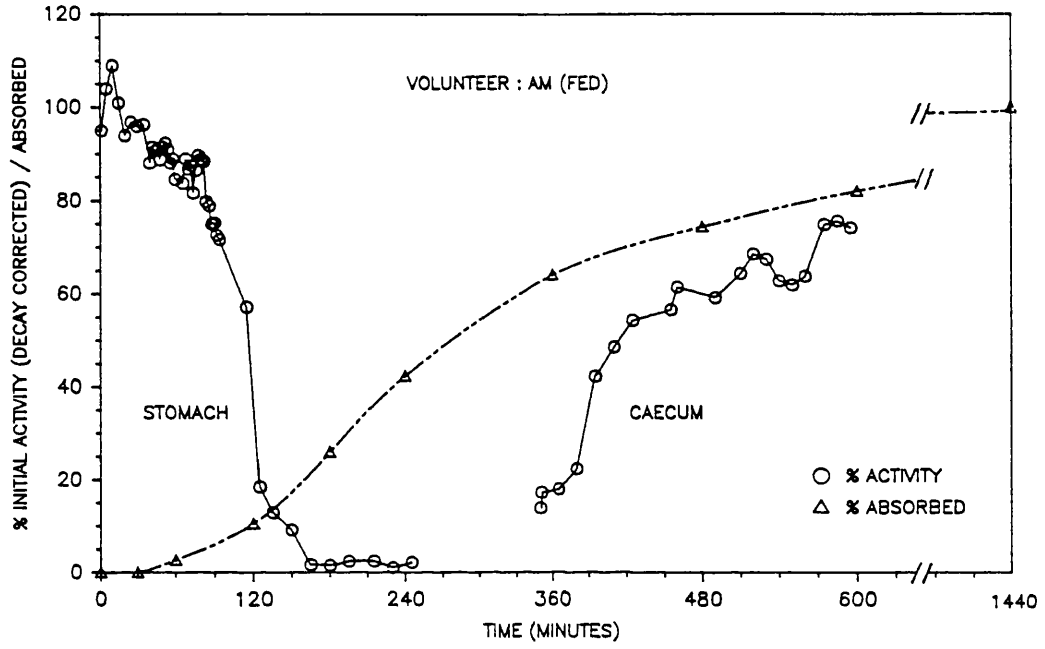


FIGURE 46 : GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER AM (FED)

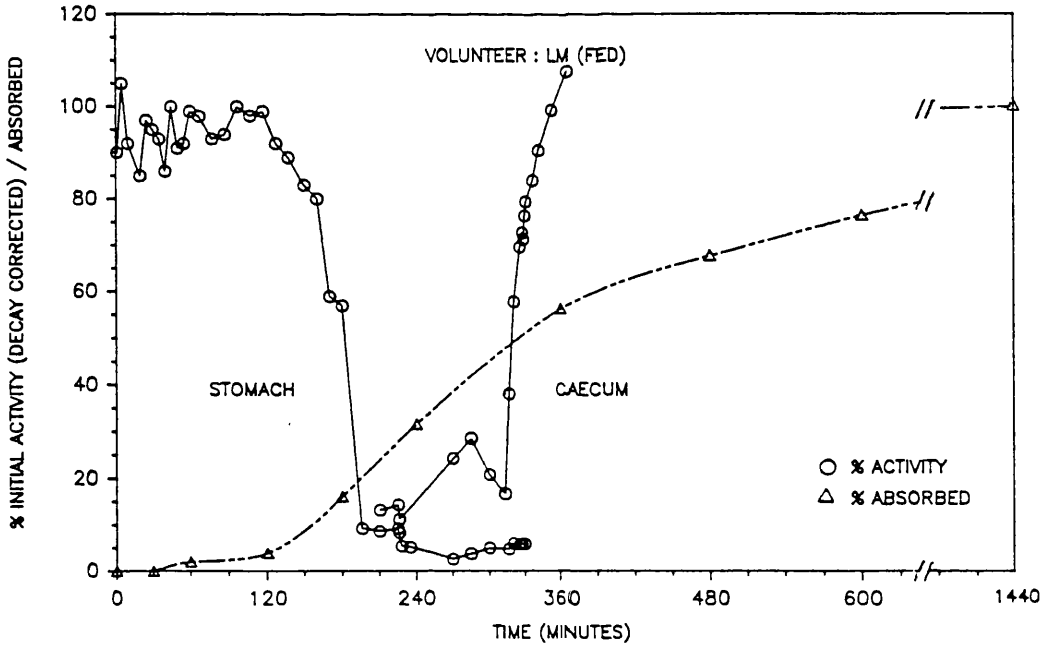


FIGURE 47 : GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS / ABSORPTION PROFILE IN VOLUNTEER LM (FED)

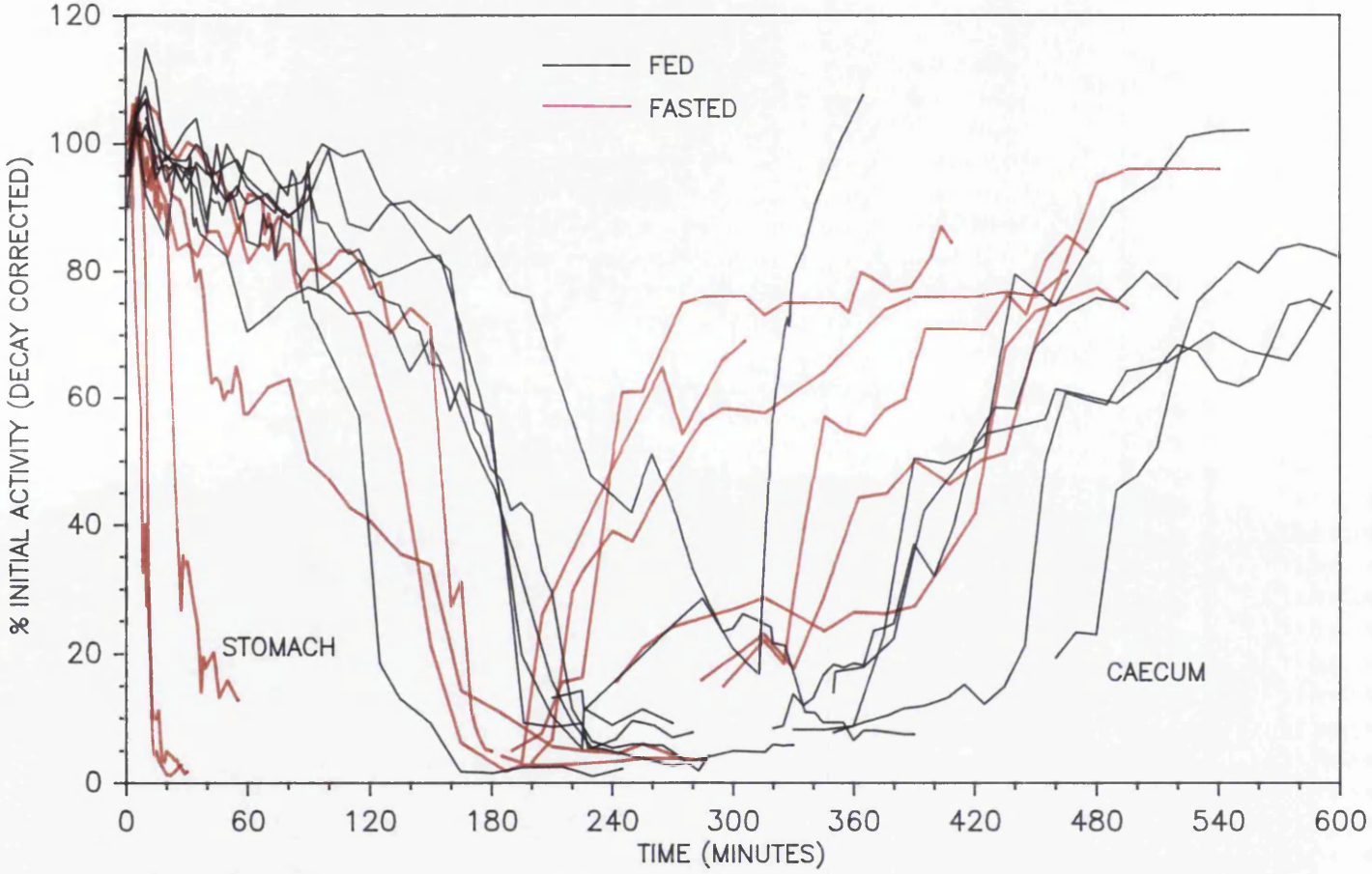


FIGURE 48 : GASTRIC EMPTYING AND CAECUM ARRIVAL OF PELLETS IN FED AND FASTED VOLUNTEERS

and exponential patterns of emptying have been reported based on the average data (*O'Reilly et al* 1987). However, such a composite curve may not be typical in shape of the individual curves, and may mask highly important variations and patterns of emptying (*Devereux* 1987; *Clarke* 1989). Therefore, a display of the individual curves will permit a better qualitative comparison between individuals and between dosage forms. A quantitative comparison can be made by focusing on some important features that can characterise the emptying pattern, and the estimated numerical values can then be analyzed statistically (*Elashoff* 1981; *Elashoff et al* 1982).

In the present study, a number of parameters were estimated from the individual gastric emptying profiles which permit a quantitative comparison between the emptying pattern under fed and fasted conditions to be made. The first parameter was the time for 50% of the pellets (activity) to be emptied from the stomach (t50% emptied), interpolated from the emptying curve. Another parameter was the time to completion of gastric emptying (tcomplete), and was taken to be the time when 10% of the activity remained in the stomach region. A third parameter, the gastric emptying lag-time, was obtained by reference to the computer images rather than the emptying curves. It was defined as the time corresponding to that image when all detectable activity appeared to remain within the stomach region of interest. This is illustrated by the example on plate 2. At 20 minutes, the activity appeared as a single source in the stomach region. In the next image at 21 minutes, there was clear evidence that some pellets had begun to pass out of the stomach. Therefore, in this example, 20 minutes was defined as the lag time. Attempts to estimate this parameter from the emptying curve were made difficult by oscillations in radioactivity, probably due to redistribution of the labelled pellets within the stomach (*Clarke* 1989). From the difference between tcomplete and the gastric emptying lag time, an emptying period was estimated as the fourth parameter.

The numerical values of the parameters for individual volunteers are given in Table 21. Wide intersubject variations were observed in the numerical values of the parameters for both food status. In general, the values in the fed state were larger than the fasted values. For each parameter, the difference between fed and fasted values was analyzed statistically using an independent (unmatched) t-test. The same statistical assumptions of the ANOVA procedure apply to the t-test (*Healy et al* 1986). All variances were tested as before and were found to be homogenous. The results of the statistical analyses are also shown in Table 21. A

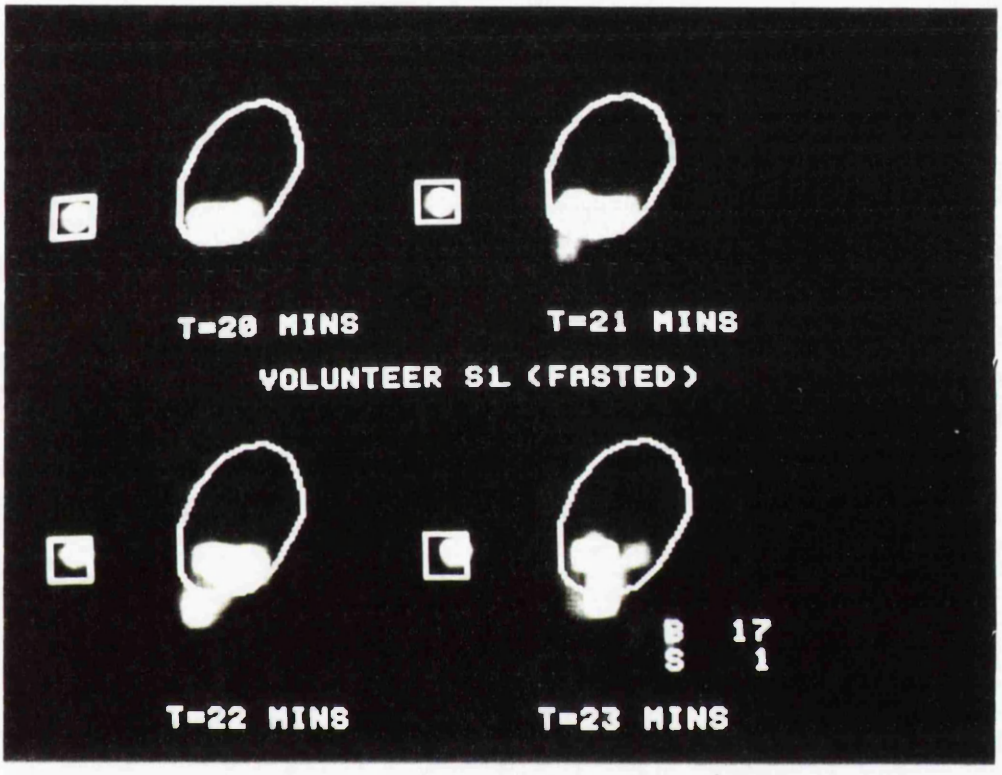


Plate 2 : GASTRIC EMPTYING OF PELLETS (start of emptying at 21 minutes)

Table 21 : NUMERICAL VALUES (MINUTES) OF THE GASTRIC EMPTYING PARAMETERS

Volunteer		Lag time		t complete		Emptying period		t50% emptied	
Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed
KI	LM	7	117	16	196	9	79	11	183
JE	KN	27	145	184	315	157	170	88	179
SL	EY	20	105	41	255	21	150	24	183
NA	AM	4	59	18	165	14	106	8	118
VK	WI	120	50	215	230	95	180	154	176
JA	WW	95	160	187	380	92	220	136	246
Mean		46	106	110	257	65	151	70	181
SEM		(20)	(18)	(38)	(32)	(24)	(21)	(27)	(17)
Independent t-test		P = 0.049		P = 0.015		P = 0.023		P = 0.005	

statistically significant difference was observed between the fed and fasted values for all the four parameters ($P < 0.05$).

The results of the statistical analyses indicate that the gastric emptying pattern was significantly different under the fed and fasted states (Table 21). The average values of all four parameters were significantly increased when the volunteers were dosed in a fed state. Therefore, food not only delayed the onset but also decreased the rate of gastric emptying, resulting in a prolonged emptying period. Devereux (1987) reported a similar increase in the fed values of t50% emptied and tcomplete, with pellets of same size range and density, but no statistical difference was observed between the fed and fasted values of lag time and emptying period. Studies by Christian *et al* (1980) and Davis *et al* (1984, 1987) also showed that, following a heavy meal, the pellets were emptied more slowly than a lightly fed state. On the other hand, the timing of the pellets administration relative to eating, was reported to have no influence on the emptying rate. Pellets dosed before, during and after a meal were shown to have similar half-lifes of emptying (*O'Reilly et al* 1987). They further reported that pellets dosed predispersed with food, emptied at the same rate as those taken in a capsule, but dispersion within the stomach was more rapid.

Whilst, it has been asserted by Beckett (1981) and Eskilson (1985) that multiunit dosage forms have the advantage of wide distribution in the stomach, the scintigraphic images obtained for both food status in the present study, revealed that the distribution was only limited. This is in contrast to the findings of Hardy *et al* (1985) and O'Reilly *et al* (1987), and may indeed be attributed to differences in the surface characteristics of the pellets studied. The anionic exchange resin beads employed by these workers were uncoated, whilst the Amberlite pellets used in the present study were coated with ethylcellulose. Thus, their ability to disperse could have been reduced by the more hydrophobic nature of the pellet surfaces (*Devereux* 1987; *Clarke* 1989). It was further observed that, in general, the pellets were emptied as a series of small boluses, an observation shared by Devereux (1987) and Clarke (1989). Nevertheless, in a number of volunteers dosed fasted, emptying occurred as a few large boluses and was complete within a short period.

The majority of gastric emptying studies (involving multiunit dosage forms), make no reference to a lag time of emptying (*Clarke* 1989). The most common parameter used to

describe the gastric emptying process was t50%. In reviewing the literature, Devereux (1987) observed vast differences in the t50% values reported between the workers, and the author ascribed the discrepancies to different physical properties of the pellets studied, such as size range and density. Some batches of pellets investigated by Devereux (1987) and Clarke (1989) were similar to those studied here. For these pellets, they reported a mean fasted t50% value of 125 minutes (range 64 - 211) and 185 minutes (range 87 - 477) respectively, compared to 70 minutes (range 8 - 154) obtained in the present study. Whilst the influence of food was not evaluated by Clarke (1989), Devereux (1987) reported a mean fed t50% value of 181 minutes (range 106 - 218), identical to that obtained here (range 118 - 246). Notwithstanding that the fed values were comparable, it appeared, even when the pellets were similar in size and density, that the gastric emptying rate (as defined by the t50% value) could vary considerably between studies. In addition, within each study itself, the results were subject to large variations between individuals, as evidenced by the wide range of values obtained.

Clarke (1989) noted that some volunteers were consistently rapid emptiers, whilst some were consistently slow emptiers. Variations within subjects have also been reported by Plankey *et al* (1988), and may be related to their emotional status, such as stress and anxiety, which affect the gastric emptying process (*Kaus and Fell* 1984). A variety of other factors may contribute to the variations obtained within and between studies, even when the dosage forms are essentially similar in their physical characteristics. In the fasted state, the stomach generally shows minimal motor activity. However, a short period of intense contractions known as the migrating motor complex (housekeeper effect), occurs every 2-3 hours and clears the stomach of fasting contents into the small intestine. Therefore, emptying of a dosage form dosed in the fasted state, is dependent on the time of administration and the time of the next housekeeper waves which occur at regular intervals in a given subject (*Cortot* 1984). Since the interval between two housekeeper contractions is highly variable from one individual to another, this will further contribute to differences between subjects.

The physiological conditions in which the pellets are exposed in the stomach under the fed mode are very different from those of the fasted state, and may explain the differences observed in gastric emptying between the two food status. The fed state is characterised by continuous contractile activity of the antrum, which mixes and grinds the stomach contents.

The rate of emptying in the fed mode varies between individuals (*Heading et al* 1971) and is also influenced by the volume, osmolality and nutrient composition of the meal (*Cortot* 1984). All these factors may thus cause variations in results between studies. The similarity of the mean fed t50% value obtained in the present study and that of Devereux (1987) is noteworthy. This is in view of the fact that the meal used in both studies was the same, and this could have contributed to the similarity of results obtained.

b) small intestinal transit and caecum arrival

Arrival of pellets at the caecum was described by a t50% value for caecum arrival, interpolated from the caecum arrival curves. This represented the time, from ingestion, until 50% of the pellets had entered the caecum. On the other hand, the small intestinal transit time (SITT), was obtained from the difference between the t50% values for caecum arrival and gastric emptying. In all the volunteers, imaging was performed over sufficient length of time to allow for more than 50% arrival of activity at the caecum. The individual results of t50% arrival and SITT are given in Table 22. The difference between the fed and fasted values of each parameter was also analyzed statistically using the independent t-test, and the results shown in the same table.

Unlike gastric emptying, the small intestinal transit times were not affected by food, consistent with the findings of Davis *et al* (1984, 1986a, 1987), Devereux (1987) and Mundy *et al* (1989). No statistically significant difference (P = 0.681) was observed between the mean fed and fasted values (of 234 and 250 minutes respectively). This is also in accord with the observations made in Figure 48. The food induced delay in gastric emptying, was associated with a corresponding delay in caecum arrival, resulting in a fairly consistent interval between the two processes for both the fed and fasted states. Therefore, the transit of the pellets in the small intestine was independent of the gastric emptying process (*Lagerlof et al* 1974; *Read* 1984; *Devereux* 1987; *Davis* 1989). Whilst limited dispersion was observed in the stomach, the pellets appeared to be better dispersed during transit along the small intestine, but the degree of dispersion was influenced by the gastric emptying rate. Thus, when the pellets were emptied rapidly, less spreading was observed. By contrast, better dispersion was obtained when the pellets were emptied slowly and regularly in small doses as suggested by Davis (1989).

Table 22 : NUMERICAL VALUES (MINUTES) OF t50% CAECUM ARRIVAL AND SMALL INTESTINAL TRANSIT TIME

Volunteer		t50% arrival		Small intestinal transit time	
Fasted	Fed	Fasted	Fed	Fasted	Fed
KI	LM	239	318	228	135
JE	KN	390	413	302	234
SL	EY	342	388	318	205
NA	AM	244	412	236	294
VK	WI	279	454	125	278
JA	WW	425	503	289	257
Mean		320	415	250	234
SEM		(32)	(25)	(29)	(24)
Independent t-test		P = 0.042		P = 0.681 (NS)	

The small intestinal transit times observed in these studies were comparable to those reported by Devereux (1987) and Clarke (1989) for pellets of similar size range and density, and those of other workers even though the pellets studied were not necessarily of the same size or density (see *Devereux* 1987). Therefore, in contrast to gastric emptying, the small intestinal transit times do not show wide variations between protocols and methods. It is interesting to note that Davis *et al* (1984a) further reported that the small bowel transit was also unaffected by the size and shape of the dosage form.

A statistically significant difference was observed between the fed and fasted values of t50% arrival at the caecum ($P < 0.05$). The mean t50% value was increased from 320 minutes in the fasted state to 415 minutes in the fed state (Table 22). Similar effects of food were reported by Davis *et al* (1984, 1987). Following a heavy breakfast, both the gastric emptying and caecum arrival times of the pellets were significantly increased compared to a lightly fed state. On the other hand, although Devereux (1987) observed that the caecum arrival times were increased by an average of 61 minutes, there was no statistical difference when compared to the fasted values.

The caecum arrival process was often characterised by accumulation of the pellets at the ileocaecal region (plate 3), followed by emptying in boluses which was quite rapid in certain cases, resulting in a sharp increase in activity in the caecum (Figures 36-47). This pattern of caecum filling has also been previously noted by Devereux (1987) and Spiller *et al* (1986). Once in the caecum, a retrograde passage of the contents was prevented by the ileocaecal valve, and the pellets exhibited only very slow movements. Although the rates of spreading and transit in the colon were reported to be highly variable (*Metcalf et al* 1987), the pellets have the potential for wide distribution (*Hardy and Perkins* 1985). Plate 4 shows the dispersion of the activity in the colon region of one of the volunteers. Wide distribution was usually observed from the images taken 24 hours after dosing, but occasionally this was also observed towards the end of the imaging studies (12 hours after dosing).

c) gastrointestinal transit and absorption

The results presented in the foregoing sections clearly indicated that food caused a significant delay in the gastric emptying of the pellets. Indeed this may explain the differences observed in the absorption characteristics of Formula A under the fed and fasted conditions. The mean

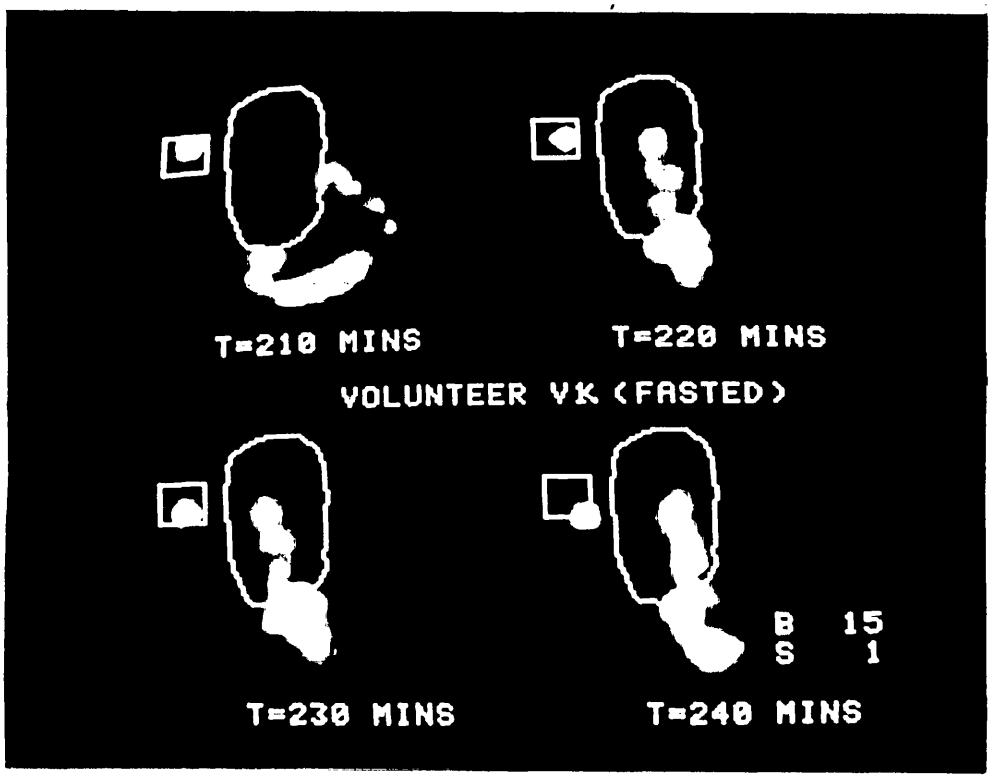


Plate 3 : CAECUM ARRIVAL OF PELLETS

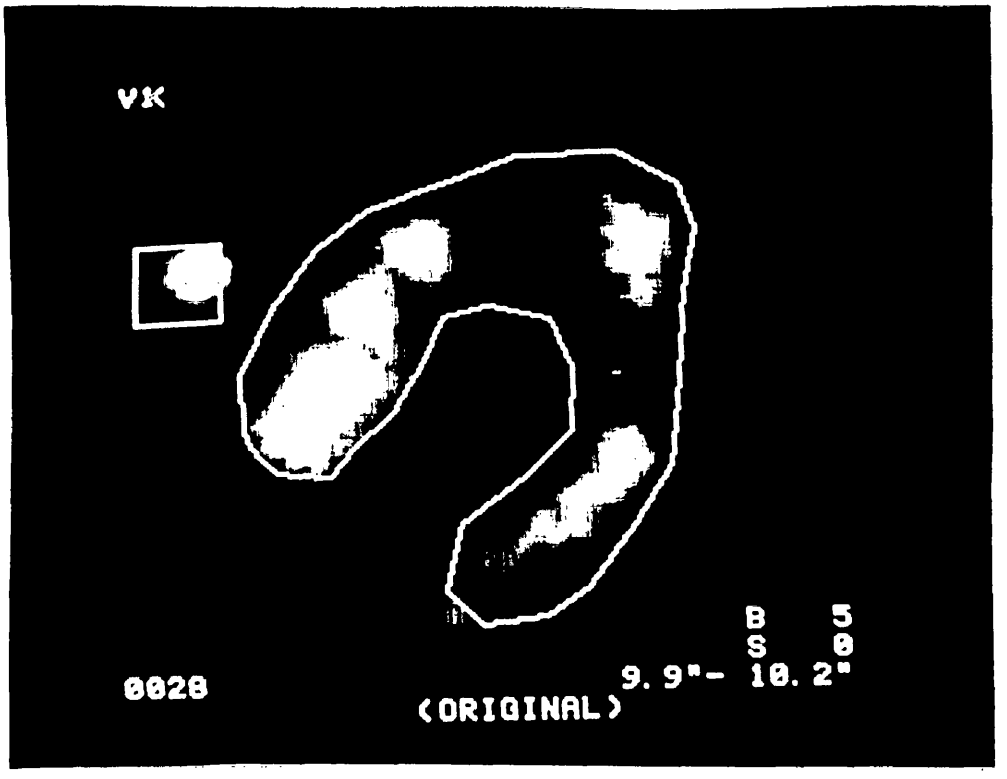


Plate 4 : DISPERSION OF PELLETS IN COLON

serum level and absorption profiles of Formula A are depicted in Figures 49 and 50 respectively, together with the distribution of the pellets in the gastrointestinal tract. The demarcated areas under the curves denote the time the pellets spent in the stomach, small intestine and colon. The two boundaries represent the respective t50% values for gastric emptying and caecum arrival of the pellets. It is interesting to note the association between a delay in the gastric emptying (and caecum arrival) of the pellets and a concomitant shift of the serum level and absorption curves to the right.

Compared to the stomach region the absorption rate appeared to be relatively faster when the pellets had emptied into the small intestine (Figure 50). This is also demonstrated in the individual absorption profiles (calculated using the Wagner-Nelson method) depicted in Figures 36 to 47, together with the gastric emptying and caecum arrival curves. Absorption was generally slow when a large proportion of pellets remained in the stomach. In contrast, a noticeable increase in absorption rate was observed when the pellets began to empty into the small intestinal region. A direct comparison of the rates of absorption in the fed and fasted conditions, indicates that food did not appreciably alter the absorption rate while the pellets were in the stomach, but merely prolonged the slow absorption phase, consequent to the delayed gastric emptying. However, because the gastric residence time was relatively brief in the fasted mode, this slow absorption phase would tend to be obscured under this condition (Figures 36, 37, 39). Therefore, a delay in gastric emptying would reasonably explain the existence of a slow absorption phase in the fed mode, which resulted in the serum level profile being shifted to the right. An absorption lag time was also observed in a number of volunteers, and could be attributed to a delay in disintegration of the capsule.

A number of studies on sustained release theophylline products (discussed in section 1.3.2) have reported a similar consequence of food effects on the rate of absorption. Some of the products evaluated were single-unit tablets while others, such as "Somophyllin - CRT" (*Pedersen and Moller-Petersen* 1985) and "Slo - Bid Gyrocaps" (*Hendeles and Weinberger* 1986) were multiunit preparations in the form of coated granules. Although the gastrointestinal transit properties of these products were not evaluated in the studies, the observed food effects could similarly be related to the delayed gastric emptying.

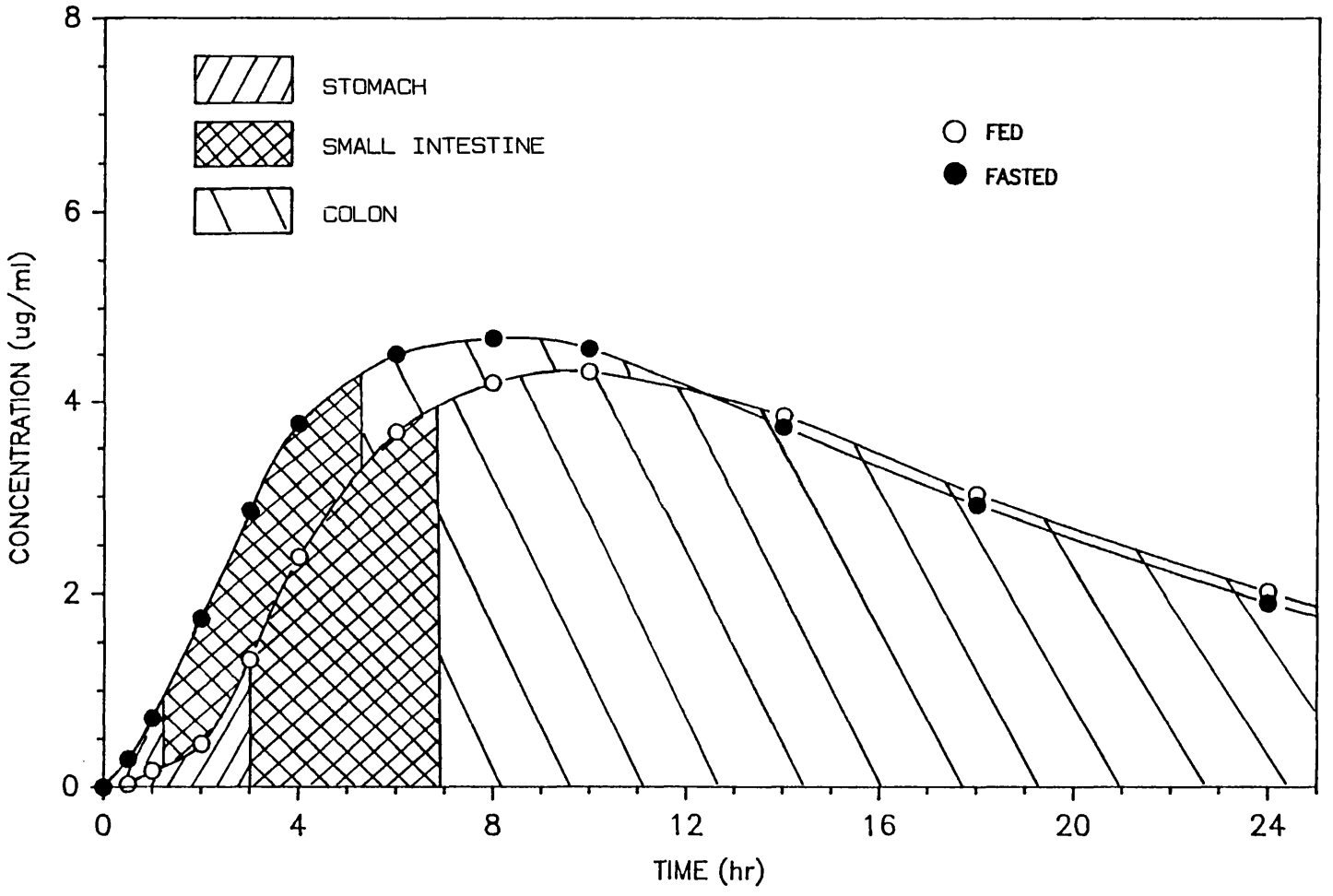


FIGURE 49 : AVERAGE SERUM THEOPHYLLINE CONCENTRATION PROFILES OF FORMULA A DOSED FED AND FASTED, AND DISTRIBUTION OF PELLETS IN THE GASTROINTESTINAL TRACT

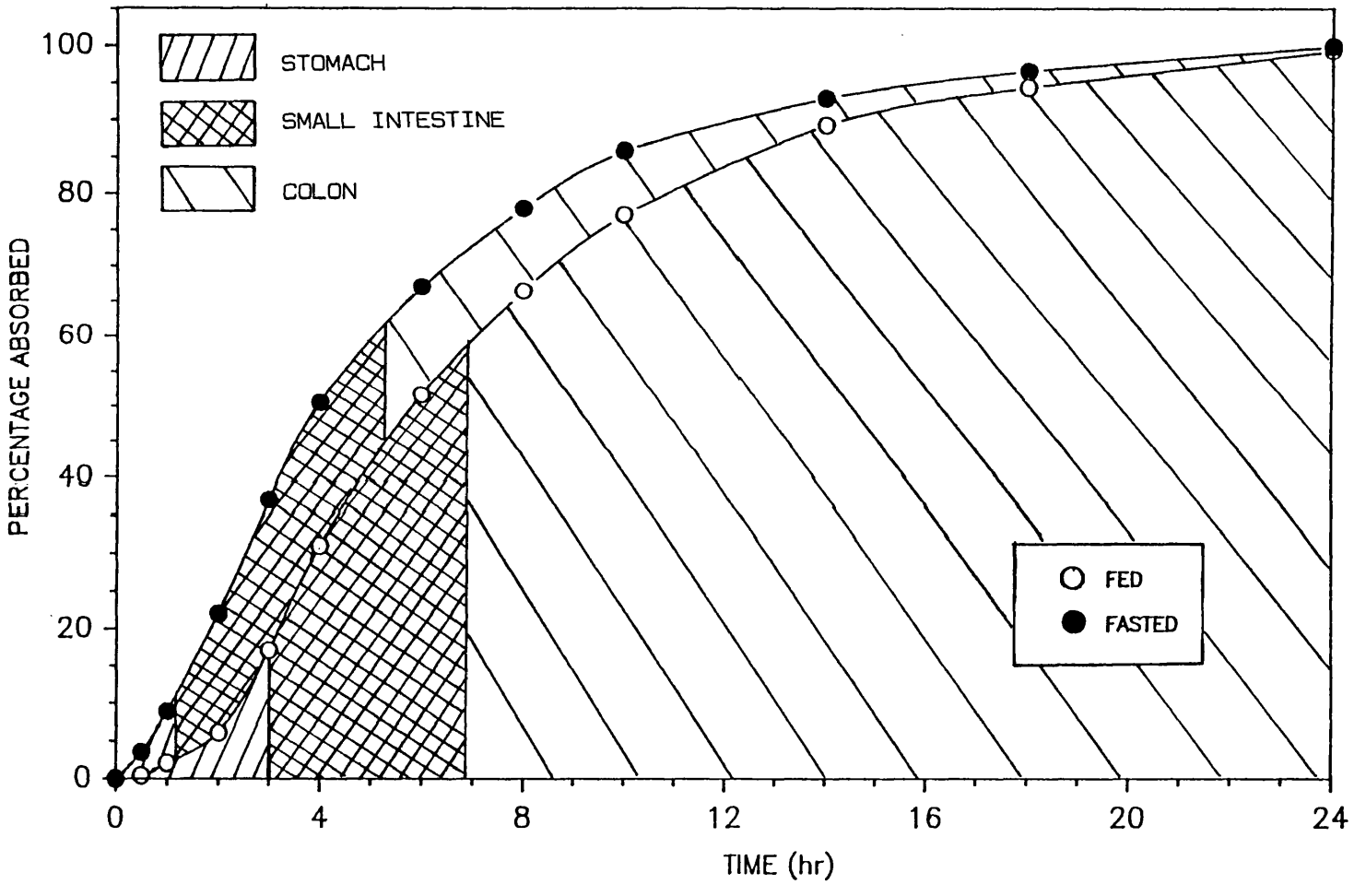


FIGURE 50 :AVERAGE PERCENTAGE ABSORBED VERSUS TIME PLOTS OF FORMULA A DOSED FED AND FASTED, AND DISTRIBUTION OF PELLETS IN THE GASTROINTESTINAL TRACT

Upon entering the small intestine, the absorption of the pellets appeared to occur at comparable rates between the fed and fasted conditions, as evidenced by the parallel nature of the two absorption curves in the small intestinal region (Figure 50). Hence, the presence of food did not influence the rate of absorption when the pellets were in the small intestine, nor was there any effect on the transit time. Bryson *et al* (1989) have investigated the effects of altering the small intestinal transit time on the absorption of a sustained release theophylline product. They reported that the bioavailability was not affected by a decrease in SITT. However, an increase in SITT resulted in a decrease in the rate of theophylline absorption, although the extent of bioavailability was unaffected.

A notable feature that can be observed in Figure 50, as well as the individual absorption profiles (Figures 36 to 47) is that, a considerable amount of drug absorption was occurring when the pellets were in the colon. This is noteworthy since the orocaecal transit time was relatively brief and the preparation was designed to release the drug over an extended time period. In both food conditions, an estimated 40% of the drug was found to be absorbed when the pellets were in the colon, over a period of approximately 16 hours (Figure 50). This is in accord with the results obtained by Sommers *et al* (1990), in which approximately 40% of the administered dose of a sustained release theophylline product was reported to be additionally absorbed in the colon. Therefore, the theory that drug absorption could be limited to a definite segment of the gastrointestinal tract, or the so-called absorption window (*Hirtz* 1984) does not apply to theophylline.

The extent of absorption when the pellets were in the different regions of the gastrointestinal tract was estimated from the individual absorption curves, and the numerical values are given in Table 23. The percentage absorbed whilst the pellets were in the stomach was estimated at t50% gastric emptying, whilst in the small intestine, was between t50% gastric emptying and t50% caecum arrival. The remaining amount constitutes the percentage absorbed in the colon. Only approximately 9% of the drug was absorbed during residence of the pellets in the stomach when fasted and 18% when fed. The latter increase could be attributed to the prolonged gastric residence time of the pellets. Comparable amounts were absorbed when the pellets were in the small intestine and colon, particularly in the fed mode. Thus, the colon constitutes an important absorption site for the oral sustained delivery of theophylline.

Table 23 : PERCENTAGE OF THEOPHYLLINE ABSORBED DURING RESIDENCE OF PELLETS AT VARIOUS REGIONS OF THE GASTROINTESTINAL TRACT

	Volunteer	Percentage absorbed		
		Stomach	Small intestine	Colon
Fasted	KI	2	58	40
	JE	4	52	44
	SL	3	72	25
	NA	2	71	27
	VK	23	25	52
	JA	17	46	37
	Average	9	54	37
Fed	LM	16	33	51
	KN	17	31	52
	EY	13	37	50
	AM	10	60	30
	WI	16	44	40
	WW	38	39	23
	Average	18	41	41
Overall average		14	47	39

The rates of absorption when the pellets were in each of the regions were approximated by dividing the percentage absorbed by the corresponding residence time, and are given in Table 24. Although this may be a crude approximation, it nevertheless permits the rate of drug availability to be compared, during the residence of the pellets in the different regions of stomach, small intestine and colon. The results were analyzed using the ANOVA procedure (split-plot) mentioned previously, with the assumption that the data were obtained according to a 2 factorial split-plot design. In this case, the treatment levels of factor B would be the different regions of the gastrointestinal tract, and factor A remained as the food status. The results of the statistical analysis shown in Table 24 indicate that the effects of food was not significant, nor was the interaction between the factors. This suggests that the absorption rate during residence of the pellets in each of the regions was not affected by food. On the other hand, a statistical significance was observed in factor B. A posteriori comparisons among the means using the Tukey's ratio (*Kirk* 1968) revealed that the absorption rates when the pellets were in the three regions were significantly different and were of the following order (fastest to slowest) : small intestine > stomach > colon.

Therefore, as expected from the individual absorption profiles, absorption was fastest when the pellets were in the small intestine, but because of the relatively brief residence time, it accounted for only approximately 40 to 50% of the drug to be absorbed. On the other hand, whilst absorption of the pellets in the colon was slow, this was compensated by a longer exposure of the pellets to this region. The slow absorption rate when the pellets were in the colon may in part be due to a lower drug concentration remaining in the dosage form. Since the drug release was basically a passive diffusion process, the rate of dissolution would thus be reduced. Other factors such as increased viscosity of the luminal contents or reduced motility, may further contribute to the slow absorption rate observed. In common with the colon, absorption from the pellets in the stomach was relatively slow compared to that in the small intestine and could be attributed to the small absorptive area. It may thus be adduced that, variations in residence time in this region was the main contributing factor for the differences observed in the serum profiles of Formula A between the fed and fasted conditions. Though this may be the case, the overall serum concentration profile remained unaffected, albeit a slight delay or shift of the curve to the right, and may not be therapeutically significant. Unlike Uniphyllin, the mechanism underlying the drug release from Formula A was not influenced by the presence of food.

Table 24 : APPROXIMATE RATE OF THEOPHYLLINE ABSORPTION (% hr^{-1}) DURING RESIDENCE OF PELLETS AT VARIOUS REGIONS OF THE GASTROINTESTINAL TRACT, AND ANOVA RESULTS

		Volunteer	Factor B		
			Stomach	Small intestine	Colon
Factor A	Fasted	KI	10.9	15.3	2.0
		JE	2.7	10.3	2.5
		SL	7.5	13.6	1.4
		NA	15.0	18.0	1.4
		VK	8.9	12.0	2.7
		JA	7.5	9.6	2.2
		Average	8.8	13.1	2.0
	Fed	LM	5.2	14.6	2.7
		KN	5.7	8.0	3.0
		EY	4.3	10.8	2.8
		AM	5.1	12.2	1.8
		WI	5.5	9.5	2.4
		WW	9.3	9.1	1.5
		Average	5.9	10.7	2.4

ANOVA table

Source	SS	df	MS	F	P
Factor A	25.0	1	25.0	3.07	0.108(NS)
Error (m. plot)	81.5	10	8.1	-	-
Factor B	566.9	2	283.5	56.83	<0.001
A X B	18.3	2	9.2	1.84	0.184(NS)
Error (s. plot)	99.8	20	5.0	-	-
Total	791.7	35	-	-	-

A number of potential sources of error have been recognised in gamma scintigraphic studies of gastric emptying (*Christian et al* 1983; *Moore et al* 1985; *Tothill et al* 1978). They include variations in attenuation of the gamma ray resulting from anteroposterior movement of the radioactive source, overlapping of the stomach with the duodenum and jejunum when activity is present in all these regions, and scatter of radiation. The first of these can be largely corrected by using the geometric mean of the counts obtained from the anterior and posterior heads of the camera (*Tothill et al* 1978). As for the second, Meyer (1984) suggested that it might be insignificant. Activity emptied from the stomach passes rapidly through the duodenum and jejunum, where overlap might be a problem. The third source proved more of a problem, particularly towards the end of the emptying period where only a very low count rate is present in the gastric region (*Clarke* 1989). For this reason, complete emptying was found to be more reliably described as being 10% of activity remaining in the stomach region rather than when no activity was recorded. Nevertheless, these errors, do not seriously impair the application of the gamma scintigraphic technique in gastrointestinal transit studies.

5.4 CONCLUSION

In conclusion, the *in-vivo* performance of the novel preparation is satisfactory under both food conditions. The results obtained from the comparative study with Uniphyllin indicate that the two preparations were bioequivalent in the fasted mode. In the fed mode however, the absorption rate of Uniphyllin was increased considerably, whilst that of Formula A was essentially unaffected, although a slight delay in initial absorption was noted. On the other hand, the extent of absorption of each preparation and between the preparations, was not significantly different under both food conditions.

The gastrointestinal transit studies of the novel preparation revealed that food caused a delay in the gastric emptying, but was without influence on the small intestinal transit time. The delayed gastric emptying was associated with a delay in drug absorption. However, this may not be therapeutically significant.

In both food conditions, absorption whilst the pellets were in the stomach was generally slow compared to that in the small intestinal region. Only a relatively small percentage of the drug was absorbed when the pellets were in the stomach (average 14%). In contrast, a considerable percentage (an average of 39%) was absorbed when the pellets had reached the colon. Thus the colon represents an important absorption site for the oral sustained delivery of theophylline. Dispersion of the pellets in the stomach was limited but appeared better dispersed in the small intestine and colon.

Chapter 6: SUMMARY AND GENERAL CONCLUSION

Satisfactory results were obtained in each of the stages of study, leading to the successful development and evaluation of the novel sustained release preparation. The subunits of the preparation, which comprised spherical drug cores of high theophylline content (80%) were successfully prepared using the extrusion/spheronisation technique. Whilst keeping the amount of excipients to a minimum, a high yield of pellets in the desired size range was achieved. The pellets which were both smooth and spherical, possessed the ideal shape for application of the rate-controlling membrane coat. A coating mixture of ethylcellulose and methylcellulose of viscosity grade 400 cp was found suitable for achieving the desired *in-vitro* release characteristics. By manipulating the coat thickness, the rate of drug release could be reliably varied. Stability of drug release as a function of storage times was assured by additional thermal treatment of the coat. In addition, the drug release was essentially pH independent.

In-vivo, the preparation produced satisfactory sustained serum profiles over 24 hours. Absorption was complete when compared to a reference solution of the drug. As reflected in the *in-vitro* studies, the *in-vivo* rate of drug release, and hence the serum concentration profile, could be varied by altering the coat thickness. A satisfactory correlation was also obtained between *in-vivo* and *in-vitro* results. The *in-vivo* performance of the novel preparation was essentially unaffected by the presence of food. It was bioequivalent to Uniphyllin when compared in the fasted condition. However, the absorption rate of the latter was considerably increased in the fed mode, although the extent of absorption between the two preparations was not significantly different.

Food however, caused a delay in the gastric emptying of the pellets, resulting in a slight delay in drug absorption, but this may not be therapeutically significant. On the other hand, the small intestinal transit times of the preparation were not affected by the presence of food. Drug absorption whilst the pellets were in the stomach was slower than when they had emptied into the small intestine by comparison, and constituted a relatively small percentage of the drug to be absorbed. A considerable percentage of the drug was absorbed when the pellets were in the colon under both food conditions, and the remaining amount whilst in the small intestine. Except for the delay in the stomach caused by delayed emptying, absorption

when the pellets were in the small intestine and colon was essentially unaffected by food.

In conclusion, the results from these single dose studies suggest that this novel multiunit formulation would produce a sustained level of theophylline when used *in-vivo*.

Chapter 7: FURTHER WORK

There are a number of areas in which further investigations may lead to a better understanding of the *in-vivo* performance of the novel preparation, or of sustained release preparations in general. These are briefly discussed as follows:

a) multiple dose studies

Whilst single dose studies are usually sufficient to establish the validity of sustained release dosage form designs, it is of interest to perform further multiple dose steady state studies, so that the optimum dosage regimen can be determined (*Lordi* 1986). This may be conducted in comparison with an established commercial preparation. Since the absorption of the novel preparation (Formula A) has been shown to occur over approximately 24 hours, a once daily dosing interval may seem appropriate. However should resources be permitting, both 12 hours and 24 hours dosing intervals would preferably be studied. Because the therapeutic range of theophylline is well defined (section 1.3), the degree of fluctuation between peak and trough steady state concentrations of each regimen, could then be used to decide on the suitability of the dosing schedules. If the serum level profiles need further adjustments, this can be accomplished by modifying the release rate of the preparation through manipulating the coat thickness.

b) influence of circadian variations

Another interesting area which may need further investigation is the *in-vivo* performance of the preparation in relation to diurnal variations. This may be conducted by comparing the bioavailability of the daytime and nighttime doses in a multiple dose study with a 12 hourly dosing interval. Alternatively, the comparison can be made using single dose studies performed at different times of the day (morning and evening). The reason for such a comparison is that circadian changes have been observed in the absorption and disposition of theophylline between these two time periods (*Decourt et al* 1982; *Bauer et al* 1984). A number of studies have also reported chronokinetic changes in the serum concentrations of sustained release preparations (*Coulthard et al* 1983; *Lesko et al* 1980; *Reinberg et al* 1987; *Scott et al* 1981).

On the other hand, exacerbation of respiratory symptoms in patients with chronic obstructive pulmonary disease, was also noted to be influenced by diurnal variations, being more frequent during the nighttime (*Smolensky and McGovern* 1985). Any significant difference between the performance of the preparation during daytime and nighttime should therefore be considered in designing the dosage regimen for optimum therapeutic control of nocturnal asthma. An unequal daytime and nighttime dose may indeed be required if a considerable difference is observed. Such a dosage regime has been suggested by Bruguerolle *et al* (1987) based on their findings with a sustained release theophylline product.

McDevitt and Taylor (1985) reported that the circadian variations in plasma theophylline concentrations were primarily due to the absorption rate being slower at night than during the day. They further reported that the reasons for this were not yet clear. In this regard, simultaneous monitoring of the gastrointestinal transit behaviour of the dosage form would be useful. The transit behaviour may be different between daytime and nighttime, because of differences in physical activities during these periods, and may explain the differences in absorption rate. The motor activity of the gut has been shown to be minimal during sleep (*Narducci et al* 1987; *Thompson et al* 1980). Posture and position of the subject would be another important consideration (*Bennett et al* 1984). Although Khosla and Davis (1986) found no significant difference in the gastric emptying of pellets between the upright and supine positions, Nimmo and Prescott (1978) reported that lying on the left hand side caused a delay in emptying. Therefore it would interesting to determine if all these could be interrelated.

c) timing of meal intake and types of food

In the present study, the effects of food were evaluated by dosing the preparation immediately after a meal. Additional studies may be carried out to investigate the gastric emptying and absorption profiles with respect to dosing of the preparation before and during the meal, or at certain time intervals before and after eating. Although O'Reilly *et al* (1987) have shown that the t50% values were similar when the pellets were administered either before, during or after a meal, it was also noted that dosing before eating resulted in an initially faster emptying rate. Hence, the timing of the drug administration with ingestion of food, may influence the gastric emptying pattern. This may in turn affect the drug absorption.

The type, size and content of the food represent other factors that need to be considered in further studies on food effects. For example, diet rich in fat content has been shown to exert a greater influence on the bioavailability of some sustained release preparations (*Brazier et al* 1989; *Karim et al* 1985a; *Tada et al* 1989). Similarly, prolongation of gastric emptying was also reported to be more pronounced with fats, compared to other types of food (*Aiache and Aiache* 1985; *Cortot* 1984). On the other hand, gastric emptying has been shown to be significantly delayed by warm fluids but accelerated by cold drinks (*Ritschel and Erni* 1977). The effects of meal temperature on the bioavailability of a sustained release product have also been investigated by Schulz *et al* (1987). They found that a hot evening meal tends to cause a decrease in the bioavailability compared to a cold one. Hence, simultaneous monitoring of the gastrointestinal transit properties and the bioavailability of the preparation would determine if such food effects on bioavailability are indeed associated with changes in the rate of gastric emptying.

d) pellet size

In section 5, it was observed that the delay in gastric emptying of the pellets in the fed mode, resulted in the drug absorption being delayed. Therefore, if the pellets can empty more rapidly, it may be possible to prevent the delay in drug absorption. Whilst it has been suggested that the critical size for pellets to empty gradually in the fed mode was 3mm (*Meyer* 1989), studies using spheres of 1-3mm also showed that the rate of emptying was influenced by the size of the spheres in this range (*Meyer et al* 1988). The larger spheres were emptied from the food-filled stomach more slowly than the smaller ones. In addition, results obtained from the earlier studies by Meyer *et al* (1979) and Mayer *et al* (1982, 1984) tended to suggest that particles of 1 mm or less would empty unimpeded like liquid contents. (*Kelly* 1981). The size range of the pellets used in the present study was 1.18-1.4 mm. Therefore, it would be interesting to study drug pellets of smaller sizes, example of approximately 0.5mm to determine if the gastric emptying and hence drug absorption would be independent of food.

e) subjects from other population groups

The volunteers used in the present study have been restricted to normal healthy males between the ages of 19 and 38 years old. This group represents only a discrete proportion of the population, and further studies ought to be perform to cover other population groups

who may differ in the gastrointestinal handling of the dosage form as well as pharmacokinetic disposition of the drug. For example, the gastrointestinal transit properties of solids or liquids have been reported to be different between the sexes (*Datz et al* 1986) and between smokers and non-smokers (*Notivol et al* 1984). On the other hand, the disposition of theophylline is influenced by age, smoking, physiological abnormalities such as hepatic dysfunction and cardiac disease and also concomitant administration of certain drugs (see section 1.3.1). Therefore, these factors should be considered for further investigations. The findings from such studies may add to our understanding of the complexity of factors that may influence the performance of sustained release preparations in general, and of the novel preparation in particular.

In addition, it would be useful to evaluate the performance of the novel preparation using patients requiring therapy with the drug. A comprehensive protocol for such an evaluation has been described in the study by Baker *et al* (1988). The volunteers were recruited from patients maintained on a sustained release theophylline product. During the trial period, this product was then substituted with the test preparation. The respiratory function of the patients was also monitored simultaneously. In this regard, patients on a single drug therapy are preferred so that the respiratory measurements will not be affected by other antiasthmatic drugs. Studies such as these will aid in determining the optimal dosage regimen of the preparation.

f) application of the sustained release system to other drugs

The results presented in this study indicated that the sustained release system which was developed for theophylline, produced satisfactory control of the drug release both *in-vitro* and *in-vivo*. It is envisaged that the system may be applied to other drug candidates, albeit with minor adjustments, such as in the pellet formula, or coat thickness to achieve the desired release profiles. Thus, the system may not only be used to develop sustained release products with other drugs, but also for studying their absorption characteristics in relation to their gastrointestinal transit properties.

Should a more potent drug be used, it is then possible to prepare pellets of lower drug loading. Under this condition, it is suggested that instead of using inert labelled pellets of similar characteristics as the radioactive marker for imaging, the drug pellets themselves

should be labelled. Even though the inert pellets can be assumed to have similar gastrointestinal transit properties as the drug pellets because of their similar characteristics, directly labelling the drug pellets will not only reduce the amount of pellets to be administered, but also remove any uncertainty regarding the accuracy of the method.

However, the existing method may not be suitable for labelling the drug pellets. This is because the soaking procedure may result in loss of a considerable amount of drug prior to administration. Although the drug loss may be minimised by modifying the pH of the labelling solution (*Davis et al* 1987), this may not be applicable to all drugs. Not the solubilities of all drugs can be significantly reduced by altering the pH of the soaking solution. Christensen *et al* (1984) described a technique for labelling of intact dosage forms without involving the soaking procedure. It requires the incorporation of a suitable stable isotope such as Iron-58 (*Christensen et al* 1984) or Barium-138 (*Parr et al* 1985), into the dosage form. Neutron bombardment of the intact preparation, converts the stable isotope to its radioactive form, which then enables the preparation to be monitored using the gamma-scintigraphic technique.

Therefore, if drug loading of the pellets is relatively low, a small amount of the stable isotopes mentioned above can be incorporated into the formulation without affecting the manufacturing process.

APPENDICES

A1 : IN VITRO RELEASE DATA OF UNCOATED 66.7% THEOPHYLLINE PELLETS AS A FUNCTION OF pH

Time (hour)	Percentage released		
	pH 7	pH 4	pH 1
0.08	17.7(0.7)	-	-
0.17	28.0(0.3)	-	-
0.25	-	31.7(0.7)	33.3(1.2)
0.33	39.7(0.5)	-	-
0.50	46.4(1.8)	44.9(0.9)	48.1(1.1)
0.75	55.8(0.6)	-	-
1.00	61.8(0.9)	59.5(1.1)	64.5(0.9)
1.50	72.6(1.0)	-	-
2.00	79.8(1.2)	76.9(1.4)	81.0(0.9)
3.00	89.9(1.3)	-	-
4.00	96.0(1.3)	94.9(1.4)	98.0(0.8)

Figures in brackets = standard deviation (n=6)

A2: IN-VITRO RELEASE DATA OF UNCOATED 75.0% THEOPHYLLINE PELLETS AS A FUNCTION OF pH

Time (hour)	percentage released		
	pH 7	pH 4	pH 1
0.08	17.3(0.4)	-	-
0.17	28.5(0.3)	-	-
0.25	-	34.7(0.8)	36.2(0.5)
0.33	40.4(0.6)	-	-
0.50	49.3(1.1)	47.4(0.3)	50.4(0.3)
0.75	58.9(1.3)	-	-
1.00	66.0(1.5)	63.5(0.9)	67.9(0.6)
1.50	77.7(1.2)	-	-
2.00	86.1(1.1)	83.5(0.7)	87.0(0.9)
3.00	95.6(1.3)	-	-
4.00	99.4(0.8)	98.2(0.8)	98.4(1.5)

Figures in brackets = standard deviation (n=6)

A3 : IN-VITRO RELEASE DATA OF UNCOATED 80.0% THEOPHYLLINE PELLETS AS A FUNCTION OF pH

Time (hour)	Percentage released		
	pH 7	pH 4	pH 1
0.08	21.0(0.4)	18.3(0.7)	18.6(0.4)
0.17	31.7(0.9)	29.0(0.4)	30.0(0.4)
0.33	46.6(0.9)	43.3(1.5)	45.1(0.7)
0.50	56.5(0.8)	53.4(0.7)	55.2(0.8)
1.00	75.4(1.6)	70.9(0.6)	74.1(0.7)
1.50	87.0(0.6)	83.0(0.5)	86.2(0.6)
2.00	94.0(0.7)	89.8(0.4)	92.0(0.5)
3.00	99.8(0.3)	99.5(0.7)	99.1(0.3)
4.00	100.0 -	100.0 -	100.0 -

Figures in brackets = standard deviation (n=6)

A4 : IN VITRO RELEASE DATA OF UNCOATED 80.0% THEOPHYLLINE PELLETS AS A FUNCTION OF STORAGE TIME

Time (hour)	Percentage released		
	initial	6 months	12 months
0.08	21.0(0.4)	18.7(0.6)	20.4(0.60)
0.17	31.7(0.9)	29.8(0.4)	32.6(0.40)
0.33	46.6(0.9)	45.1(0.4)	46.1(1.0)
0.50	56.5(0.8)	54.9(0.3)	56.4(0.7)
1.00	75.4(1.6)	72.0(0.5)	74.1(0.7)
1.50	87.0(0.62)	85.0(0.5)	86.6(0.9)
2.00	94.1(0.7)	92.3(0.4)	93.8(0.7)
3.00	99.8(0.3)	99.9(0.2)	99.5(0.4)
4.00	100.0	100.0	100.0

Figures in brackets = standard deviation (n=6)

A5 : IN VITRO THEOPHYLLINE RELEASE DATA OF ETHYLCELLULOSE COATED PELLETS AS A FUNCTION OF COAT THICKNESS, EXPRESSED AS THEORETICAL WEIGHT GAIN (TWG)

Time (hour)	Percentage Released						
	TWG=1.9%	TWG=2.5%	TWG=3.8%	TWG=5.0%	TWG=7.5%	TWG=10.0%	TWG=12.5%
0.5	26.0(1.9)	9.2(0.8)	1.0(0.1)	0.18(0.02)	-	0.20(0.02)	0.15(0.04)
1.0	44.5(3.0)	17.1(1.5)	1.9(0.2)	0.27(0.02)	0.25(0.04)	0.25(0.02)	0.21(0.08)
2.0	64.2(3.9)	30.1(2.3)	3.6(0.3)	0.47(0.03)	0.43(0.04)	0.39(0.06)	0.36(0.13)
3.0	74.0(3.8)	38.8(2.7)	5.1(0.4)	0.70(0.04)	0.60(0.05)	-	0.48(0.17)
4.0	79.1(3.8)	45.5(2.9)	6.4(0.5)	0.92(0.05)	0.77(0.05)	0.70(0.07)	0.60(0.19)
6.0	83.6(3.1)	54.3(2.8)	8.8(0.6)	1.35(0.06)	1.11(0.07)	0.99(0.09)	0.89(0.23)
8.0	86.1(2.6)	59.0(2.4)	10.8(0.7)	1.75(0.08)	1.44(0.08)	1.26(0.11)	1.12(0.23)
10.0	87.5(2.6)	61.9(2.6)	12.6(0.9)	2.16(0.09)	1.77(0.10)	1.53(0.13)	1.31(0.23)
12.0	89.0(2.5)	63.8(2.7)	15.1(0.9)	2.54(0.11)	2.09(0.12)	1.77(0.16)	1.59(0.24)

Figures in brackets = standard deviation (n=6)

A6 : IN VITRO THEOPHYLLINE RELEASE DATA OF ETHYLCELLULOSE COATED PELLETS WITH DIFFERENT COATING ADDITIVES (THEORETICAL WEIGHT GAIN OF ETHYLCELLULOSE = 12.5%)

Time (hour)	Percentage Released						
	No Coating Additive	Acacia [0.4]	PEG400 [0.8]	NaCl [0.4]	MC15 [0.32]	MC400 [0.16]	PEG4000 [0.8]
0.5	0.15(0.04)	0.46(0.02)	0.22(0.06)	0.38(0.20)	1.01	0.35(0.09)	2.43(0.41)
1.0	0.21(0.08)	0.68(0.01)	0.47(0.09)	0.81(0.25)	2.08(0.05)	0.84(0.13)	3.91(0.82)
2.0	0.36(0.13)	1.08(0.03)	1.02(0.13)	1.94(0.30)	4.06(0.08)	2.26(0.20)	7.26(1.63)
3.0	0.48(0.17)	1.39(0.03)	1.56(0.17)	3.28(0.31)	5.65(0.12)	4.00(0.25)	10.64(2.57)
4.0	0.60(0.19)	1.69(0.04)	2.13(0.20)	4.71(0.32)	7.12(0.10)	5.83(0.27)	14.03(3.73)
6.0	0.89(0.23)	2.24(0.06)	3.26(0.25)	7.10(0.34)	9.91(0.18)	9.60(0.31)	20.52(3.82)
8.0	1.12(0.23)	2.73(0.07)	4.47(0.30)	8.93(0.31)	12.53(0.28)	13.81(0.37)	27.21(6.12)
10.0	1.31(0.23)	3.20(0.08)	5.63(0.33)	10.59(0.30)	15.11(0.30)	17.81(0.73)	32.61(7.66)
12.0	1.59(0.24)	3.65(0.10)	6.74(0.35)	12.07(0.28)	17.42(0.42)	22.01(0.41)	39.12(8.95)

Figures in ( ) = standard deviation (n=6)

Figures in [ ] = coating additive to ethylcellulose ratio

A7 : IN VITRO THEOPHYLLINE RELEASE DATA OF ETHYLCELLULOSE COATED PELLETS WITH DIFFERENT COATING ADDITIVES (THEORETICAL WEIGHT GAIN OF ETHYLCELLULOSE = 5%)

Time (hour)	Percentage Released					
	No Coating Additive	NaCl[0.4]	Acacia[0.4]	MC15[0.32]	MC400[0.16]	PEG4000[0.8]
0.5	0.18(0.02)	2.21(0.18)	3.5(0.7)	4.1(0.1)	1.4(0.1)	6.3(0.4)
1.0	0.27(0.02)	4.53(0.45)	6.8(1.2)	7.4(0.3)	3.6(0.1)	12.4(1.0)
2.0	0.47(0.03)	8.32(0.98)	12.4(2.3)	14.2(0.6)	9.1(0.2)	22.9(1.8)
3.0	0.70(0.04)	11.71(1.32)	17.1(2.5)	19.6(0.6)	14.6(0.3)	31.6(2.2)
4.0	0.92(0.05)	15.16(2.0)	21.3(3.2)	24.5(0.9)	20.3(0.4)	39.5(2.1)
6.0	1.35(0.06)	19.82(2.26)	27.6(3.8)	33.2(1.4)	31.9(0.6)	51.5(2.8)
8.0	1.75(0.08)	24.21(2.67)	32.6(3.9)	40.2(1.5)	43.4(0.7)	60.5(2.9)
10.0	2.16(0.09)	28.02(3.00)	36.2(4.0)	46.7(1.8)	53.8(0.7)	67.6(2.7)
12.0	2.54(0.11)	31.09(2.90)	39.9(3.9)	52.7(2.2)	64.8(0.7)	72.8(2.4)

Figures in ( ) = standard deviation (n=6)

Figures in [ ] = coating additive to ethylcellulose ratio

A8 : IN VITRO THEOPHYLLINE RELEASE DATA OF COATED PELLETS COMPARING PEG 4000 AND METHYLCELLULOSE 400 AS COATING ADDITIVE

Time (hour)	Percentage Released				
	PEG 4000 TWG=12.5%	PEG 4000 TWG=7.5%	PEG 4000 TWG=5.0%	MC 400 TWG=5.0%	MC 400 TWG=4.3%
0.5	2.4(0.4)	2.8(0.2)	6.3(0.4)	1.4(0.1)	3.6(0.1)
1.0	3.9(0.8)	6.1(1.6)	12.4(1.1)	3.6(0.1)	8.6(0.2)
2.0	7.2(1.6)	11.0(0.7)	22.9(1.8)	9.1(0.2)	19.8(0.4)
3.0	10.6(2.5)	15.9(0.6)	31.6(2.2)	14.6(0.3)	30.1(0.5)
4.0	14.0(3.7)	20.4(1.2)	39.5(2.1)	20.3(0.4)	40.2(0.4)
6.0	20.5(3.8)	28.4(1.6)	51.5(2.8)	31.9(0.6)	57.4(0.7)
8.0	27.2(6.1)	36.5(1.9)	60.5(2.9)	43.4(0.7)	72.3(0.6)
10.0	32.6(7.6)	43.2(2.2)	67.6(2.7)	53.8(0.7)	85.5(0.7)
12.0	39.1(8.9)	49.6(2.4)	72.8(2.4)	64.8(0.7)	94.6(0.7)

Figures in brackets = standard deviation (n=6)

TWG = theoretical weight gain of ethylcellulose.

A9 : IN-VITRO THEOPHYLLINE RELEASE DATA OF ETHYLCELLULOSE-METHYLCELLULOSE COATED PELLETS AS A FUNCTION OF COAT THICKNESS

Time (hour)	Percentage Released					
	TWG=2.3%	TWG=2.9%	TWG=3.5%	TWG=4.1%	TWG=4.7%	TWG=5.8%
0.5	17.6(0.6)	9.4(0.6)	6.9(0.2)	4.8(0.2)	3.6(0.2)	2.3(0.2)
1.0	33.2(0.9)	21.5(1.4)	16.5(0.7)	12.1(0.3)	9.9(0.4)	7.5(0.5)
1.5	47.2(1.1)	-	-	-	-	-
2.0	58.7(1.1)	42.0(2.2)	34.0(1.1)	27.1(0.7)	22.0(0.8)	18.2(1.3)
3.0	77.0(1.2)	59.5(2.7)	50.0(1.4)	41.2(1.1)	34.2(0.9)	29.0(1.1)
4.0	89.4(1.3)	74.4(2.2)	65.4(1.7)	54.1(1.3)	45.9(1.2)	39.4(1.8)
6.0	99.1(0.3)	92.8(1.6)	85.2(1.9)	75.6(1.6)	66.4(1.3)	58.1(2.0)
8.0	100	99.6(0.6)	98.0(0.8)	91.6(1.5)	83.3(1.7)	75.0(2.5)
10.0	-	100	100 (0.3)	99.2(0.6)	95.4(0.9)	88.8(2.2)
12.0	-	-	-	100	99.9(0.2)	97.0(1.9)

Figures in brackets = standard deviation (n=6)

TWG = theoretical weight gain of coat.

A10 : IN VITRO THEOPHYLLINE RELEASE DATA OF DIFFERENT BATCH OF FINISHED PRODUCTS

Time (hour)	Percentage released							
	TWG = 2.3%		TWG = 3.5%		TWG = 4.1%		TWG = 4.7%	
	≠ 1	≠ 2	≠ 1	≠ 2	≠ 1	≠ 2	≠ 1	≠ 2
0.5	17.6(0.6)	15.0(0.6)	6.9(0.2)	7.6(0.2)	4.8(0.2)	6.6(0.5)	3.6(0.2)	5.0(0.3)
1.0	33.2(0.9)	29.3(0.5)	16.5(0.7)	15.8(0.6)	12.1(0.3)	14.2(0.2)	9.9(0.4)	11.2(0.3)
2.0	58.7(1.1)	52.5(0.6)	34.0(1.1)	32.1(1.1)	27.1(0.7)	29.1(0.5)	22.0(0.8)	24.5(0.6)
3.0	77.0(1.2)	70.3(1.3)	50.0(1.4)	47.5(1.1)	41.2(1.1)	43.3(1.1)	34.2(0.9)	37.0(0.9)
4.0	89.4(1.3)	83.6(0.9)	65.4(1.7)	61.6(1.0)	54.1(1.3)	55.8(0.8)	45.9(1.2)	48.1(1.3)
6.0	99.1(0.3)	98.0(0.8)	85.2(1.9)	81.9(1.6)	75.6(1.6)	77.1(1.3)	66.4(1.3)	69.1(1.6)
8.0	100.0	100.0	98.0(0.8)	95.0(1.2)	91.6(1.5)	92.8(1.1)	83.3(1.7)	86.1(1.8)
10.0	-	-	100.0	99.5(0.6)	99.2(0.6)	99.5(1.1)	95.4(0.9)	97.3(0.5)
12.0	-	-	-	-	100.0	-	99.9(0.2)	99.8(0.3)

Figures in brackets = standard deviation (n=6)

TWG = theoretical weight gain of coat.

All : IN VITRO THEOPHYLLINE RELEASE DATA OF ETHYLCELLULOSE-METHYLCELLULOSE COATED PELLETS AS A FUNCTION OF pH

Time (hour)	Percentage released					
	TWG = 2.3% (Formula B)			TWG = 4.1% (Formula A)		
	pH 7	pH 4	pH 1	pH 7	pH 4	pH 1
0.5	16.5(0.5)	15.5(0.4)	14.9(0.4)	5.0(0.4)	3.9(0.3)	3.4(0.3)
1.0	32.6(0.8)	34.5(0.6)	30.3(0.7)	12.5(0.4)	11.3(0.5)	11.2(0.3)
1.5	46.7(1.0)	-	43.7(1.0)	-	-	-
2.0	58.0(1.1)	60.4(0.8)	55.7(0.8)	28.4(0.7)	26.8(0.4)	27.5(0.5)
3.0	77.8(1.2)	79.3(1.2)	74.6(0.9)	43.0(0.7)	40.0(1.0)	42.6(0.3)
4.0	88.7(1.4)	90.6(1.1)	88.0(1.0)	56.2(1.0)	53.0(1.0)	56.3(0.4)
6.0	99.2(0.4)	98.3(0.7)	99.0(0.5)	77.1(1.0)	74.0(1.1)	76.7(1.2)
8.0	100.0 -	100.0 -	100.0 -	92.7(0.5)	89.6(1.5)	94.5(0.6)
10.0	-			99.1(0.6)	98.5(1.6)	99.5(0.7)
12.0	-			100.0 -	100.0 -	100.0 -

Figures in brackets = standard deviation (n=6)

A12a : IN VITRO THEOPHYLLINE RELEASE DATA OF COATED PELLETS WITH NO ADDITIONAL COAT CURING AS A FUNCTION OF STORAGE TIME.

Time (hour)	Percentage released					
	TWG = 2.3%		TWG = 2.9%		TWG = 4.1%	
	initial	6 months	initial	6 months	initial	6 months
0.5	17.6(0.6)	21.7(0.3)	9.4(0.6)	22.4(0.5)	4.8(0.2)	8.0(0.2)
1.0	33.2(0.9)	42.9(0.4)	21.5(1.4)	41.1(0.4)	12.1(0.3)	16.5(0.2)
1.5	47.2(1.1)	-	-	-	-	-
2.0	58.7(1.1)	71.1(0.8)	42.0(2.2)	66.1(1.3)	27.1(0.7)	32.9(0.4)
3.0	77.0(1.2)	88.1(1.0)	59.5(2.7)	81.9(1.3)	41.2(1.1)	48.5(0.9)
4.0	89.4(1.3)	96.6(0.6)	74.4(2.2)	90.5(0.6)	54.1(1.3)	63.0(1.0)
6.0	99.1(0.3)	99.5(0.6)	92.8(1.6)	97.8(0.8)	75.6(1.6)	82.7(1.1)
8.0	100.0	100.0	99.6(0.6)	99.1(0.3)	91.6(1.5)	95.9(0.9)
10.0			100.0	100.0	99.2(0.6)	99.4(1.3)
12.0					100.0	100.0

Figures in brackets = standard deviation (n=6)

A12b : IN VITRO THEOPHYLLINE RELEASE DATA OF COATED PELLETS WITH NO ADDITIONAL COAT CURING AS A FUNCTION OF STORAGE TIME.

Time (hour)	Percentage released			
	TWG = 4.7%		TWG = 5.8%	
	initial	6 months	initial	6 months
0.5	3.6(0.2)	8.6(0.8)	2.3(0.2)	5.8(0.4)
1.0	9.9(0.4)	18.2(0.8)	7.5(0.5)	12.6(0.4)
2.0	22.0(0.8)	37.7(2.0)	18.2(1.3)	25.9(0.5)
3.0	34.2(0.9)	52.3(1.7)	29.0(1.1)	38.3(0.7)
4.0	45.9(1.2)	65.4(2.4)	39.4(1.8)	50.4(1.2)
6.0	66.4(1.3)	85.9(2.4)	58.1(2.0)	71.0(1.4)
8.0	83.3(1.7)	96.7(1.2)	75.0(2.5)	87.8(1.0)
10.0	95.4(0.9)	99.0(0.8)	88.8(2.2)	97.0(1.1)
12.0	99.9(0.2)	99.5(0.8)	97.0(1.9)	100.0 -

Figures in brackets = standard deviation (n=6)

A13a : IN VITRO THEOPHYLLINE RELEASE DATA OF COATED PELLETS AFTER COAT CURING AS A FUNCTION OF STORAGE TIME

Time (hour)	Percentage released					
	TWG = 2.3%			TWG = 2.9%		
	initial	6 months	12 months	initial	6 months	12 months
0.5	17.5(0.3)	19.1(0.2)	18.6(0.3)	8.1(0.7)	10.5(0.5)	9.1(0.6)
1.0	35.9(0.5)	36.8(0.6)	35.7(0.7)	21.3(1.2)	24.4(0.7)	21.9(1.2)
2.0	61.6(0.6)	62.2(0.8)	61.2(0.9)	42.5(2.0)	47.0(0.8)	44.4(1.7)
3.0	79.8(0.9)	78.6(0.4)	77.6(0.9)	61.5(2.3)	65.0(1.2)	61.8(1.7)
4.0	90.9(1.2)	90.1(1.4)	90.0(0.8)	75.9(3.0)	78.6(1.0)	76.3(2.0)
6.0	99.3(0.8)	98.9(1.9)	99.0(0.6)	93.5(1.6)	95.6(1.0)	93.2(1.1)
8.0	100.0	100.0	100.0	99.6(0.3)	99.1(1.3)	99.4(0.4)
10.0				100.0	100.0	100.0
12.0						

Figures in brackets = standard deviation (n=6)

A13b : IN VITRO THEOPHYLLINE RELEASE DATA OF COATED PELLETS AFTER COAT CURING AS A FUNCTION OF STORAGE TIME

Time (hour)	Percentage released					
	TWG = 4.1%			TWG = 4.7%		
	initial	6 months	12 months	initial	6 months	12 months
0.5	4.8(0.5)	4.6(0.3)	4.2(0.3)	4.6(1.1)	3.1(0.1)	3.2(0.3)
1.0	12.6(0.6)	12.5(0.4)	13.0(0.3)	10.4(0.3)	9.4(0.2)	10.1(0.4)
2.0	27.4(0.8)	28.1(0.7)	29.0(0.8)	23.6(0.6)	22.3(0.2)	24.6(0.6)
3.0	41.8(0.9)	43.8(0.6)	45.1(0.6)	35.6(0.9)	35.4(0.3)	38.0(0.8)
4.0	54.8(0.8)	56.9(1.0)	58.5(1.0)	48.0(1.3)	47.0(0.3)	50.5(0.8)
6.0	77.1(1.0)	78.9(0.7)	81.0(1.2)	69.0(2.2)	68.3(0.3)	71.5(0.9)
8.0	92.1(0.5)	93.5(0.7)	95.1(0.9)	86.0(1.9)	84.0(0.3)	88.2(1.2)
10.0	99.5(0.4)	99.4(0.5)	100.0	96.9(0.5)	95.0(0.2)	98.1(0.8)
12.0	100.0	100.0	100.0	100.0	100.0	100.0

Figures in brackets = standard deviation (n=6)

A14

VOLUNTEER CONSENT FORM

This study is to investigate the absorption of a long acting theophylline formulation after oral administration. Theophylline is a drug that has been extensively used for treating reversible airway obstructive diseases such as asthma. Its use in a long acting form has been shown to improve its effectiveness in treating such diseases.

Two preparations based on the above formulation, but with different rates of absorption will be compared with a solution of the drug. They will be administered on three separate occasions of at least one week apart. The dose of each preparation is 250mg. During each drug administration, fourteen 5ml blood samples will be taken via a small cannula sited in a vein in the forearm.

I have read and understood the nature of this experimental study and hereby give my consent to participate.

Volunteer	Witness
Name: _____	Name: _____
Signature: _____	Signature: _____
Date: _____	Date: _____

A15a : SERUM THEOPHYLLINE CONCENTRATIONS (ug/ml) OF INDIVIDUAL VOLUNTEERS AFTER ADMINISTRATION OF A SOLUTION (DOSE = 250 mg)

Volunteer / Time (hour)	1	2	3	4	5	6	7	8	9	10	11	12	Average	SEM n=12
0.167	4.51	3.60	2.02	3.77	6.29	4.74	2.45	2.00	4.25	3.65	3.54	1.27	3.51	0.40
0.333	9.07	3.77	3.12	5.49	7.64	6.42	5.85	10.31	7.98	8.42	6.27	4.59	6.58	0.63
0.667	7.72	5.34	4.62	5.99	9.65	7.30	7.13	9.37	8.09	8.44	7.32	8.10	7.42	0.44
1.000	6.89	6.36	7.25	6.86	9.95	6.86	7.88	8.58	9.05	7.99	7.57	8.84	7.84	0.31
2.000	6.58	8.90	7.57	7.85	8.92	6.25	7.99	7.09	6.68	7.39	7.38	6.97	7.46	0.25
3.000	6.26	8.50	6.61	7.43	8.04	5.62	7.05	6.45	6.05	6.13	6.92	7.21	6.86	0.25
4.000	5.45	8.33	6.35	6.49	8.03	5.29	6.82	6.37	5.83	5.59	6.35	5.81	6.39	0.28
6.000	4.21	7.11	5.32	5.31	6.69	4.59	5.91	5.48	5.04	4.62	5.47	4.95	5.39	0.25
8.000	3.58	6.11	4.66	5.04	5.99	4.32	5.26	5.14	4.36	4.37	4.64	4.08	4.79	0.22
10.000	3.07	5.28	4.15	3.73	5.71	3.89	4.90	4.29	3.76	3.21	4.09	3.54	4.13	0.23
14.000	1.84	3.72	2.54	3.36	4.26	2.98	4.06	3.68	2.87	2.38	2.78	2.07	3.05	0.23
18.000	1.34	2.66	2.38	1.84	3.32	2.34	3.32	2.68	2.31	1.61	2.01	1.67	2.29	0.18
24.000	0.86	1.69	1.54	1.21	2.38	1.82	2.63	2.05	1.65	0.97	1.34	1.17	1.61	0.16

S.E.M = standard error of the mean

A15b : SERUM THEOPHYLLINE CONCENTRATIONS (ug/ml) OF INDIVIDUAL VOLUNTEERS AFTER ADMINISTRATION OF FORMULA A (DOSE = 250 mg)

Volunteer / Time (hour)	1	2	3	4	5	6	7	8	9	10	11	12	Average	SEM
0.50	0.13	-	-	-	-	0.07	-	0.70	-	-	0.60	-	0.24	0.09
1.00	0.54	-	-	0.67	-	0.59	0.72	1.27	0.95	0.02	1.12	0.56	0.54	0.13
1.50	0.86	0.45	0.75	1.22	0.70	1.41	1.48	1.75	1.71	0.87	1.72	1.26	1.18	0.13
2.00	1.50	1.17	1.30	1.79	1.32	2.06	1.57	2.30	2.39	1.11	2.39	2.17	1.76	0.14
3.00	1.96	2.83	2.23	3.17	1.96	2.72	3.10	3.30	3.27	2.15	3.47	3.06	2.77	0.16
4.00	2.64	4.02	3.09	3.56	2.82	3.57	3.79	3.87	3.91	3.84	3.92	3.34	3.53	0.13
6.00	3.67	5.62	4.37	4.54	4.10	3.79	4.84	4.66	4.52	3.69	4.80	3.50	4.36	0.18
8.00	3.83	6.43	5.09	4.82	4.64	3.80	5.22	4.74	4.72	3.79	4.75	3.96	4.63	0.22
10.00	3.62	6.81	4.98	4.28	5.40	3.61	5.29	4.85	4.28	4.22	4.82	3.90	4.67	0.26
14.00	2.65	5.73	4.80	3.88	5.68	3.47	4.29	4.25	3.70	3.03	4.20	3.49	4.10	0.27
18.00	1.62	4.35	3.97	3.25	5.09	2.74	3.37	3.58	2.91	3.14	3.36	2.74	3.34	0.25
24.00	0.79	3.35	2.96	2.26	3.80	1.77	2.58	2.85	2.10	2.23	2.38	1.75	2.40	0.23
36.00	0.13	1.51	1.45	1.13	2.08	0.78	1.31	1.86	0.99	0.90	1.16	0.83	1.18	0.15

S.E.M = standard error of the mean

A15c : SERUM THEOPHYLLINE CONCENTRATIONS (ug/ml) OF INDIVIDUAL VOLUNTEERS AFTER ADMINISTRATION OF FORMULA B (DOSE = 250 mg)

Time (hour) \ Volunteer	1	2	3	4	5	6	7	8	9	10	11	12	Average	SEM
0.50	0.33	-	0.05	0.70	-	0.23	0.90	1.34	0.17	0.60	1.00	0.71	0.50	0.13
1.00	1.81	0.49	0.83	1.18	0.28	0.67	1.98	1.81	1.34	1.23	1.90	1.02	1.21	0.17
1.50	2.56	1.58	1.79	2.11	0.77	1.59	3.30	2.70	2.25	1.83	2.93	2.11	2.13	0.20
2.00	2.98	2.35	2.59	2.97	1.21	2.18	3.93	3.52	2.90	2.69	3.88	2.68	2.82	0.22
3.00	4.69	3.50	3.93	5.05	2.78	3.41	6.13	4.79	3.96	3.18	4.64	3.93	4.17	0.27
4.00	4.65	4.40	4.64	5.03	4.40	4.08	6.53	5.08	4.54	3.87	4.81	4.54	4.71	0.20
6.00	4.36	6.61	5.48	5.24	6.13	4.23	6.66	4.82	4.91	4.23	5.16	4.57	5.20	0.25
8.00	3.73	7.85	5.14	5.13	6.71	4.01	6.47	5.19	4.91	4.40	4.89	4.57	5.25	0.34
10.00	3.27	7.79	4.69	4.06	6.45	3.78	5.76	5.34	4.12	4.36	4.53	3.67	4.82	0.38
14.00	2.31	5.92	3.96	2.70	5.21	3.17	4.42	4.46	3.27	3.08	3.57	2.81	3.74	0.31
18.00	1.43	4.55	3.16	2.06	3.75	2.62	3.46	3.60	2.64	2.18	2.69	1.81	2.83	0.26
24.00	0.84	2.96	2.70	1.64	2.99	1.69	2.51	2.60	1.74	1.52	1.72	1.28	2.02	0.20
36.00	0.35	1.27	1.21	0.67	1.28	0.77	1.19	1.88	0.93	0.55	0.75	0.64	0.96	0.12

S.E.M = standard error of the mean

A16 : IN VITRO THEOPHYLLINE RELEASE DATA OF FORMULA A AND UNIPHYLLIN AS A FUNCTION OF pH

Time (hour)	Percentage released					
	Formula A			Uniphyllin		
	pH 7	pH 4	pH 1	pH 7	pH 4	pH 1
0.5	5.0(0.4)	3.9(0.3)	3.4(0.3)	8.1(0.6)	7.2(0.5)	7.5(0.6)
1.0	12.5(0.4)	11.3(0.5)	11.2(0.3)	12.8(0.9)	12.1(0.6)	13.0(0.6)
2.0	28.4(0.7)	26.8(0.4)	27.5(0.5)	21.0(1.0)	20.4(0.8)	22.3(1.0)
3.0	43.0(0.7)	40.0(1.0)	42.6(0.3)	28.8(1.1)	27.7(1.0)	32.0(1.4)
4.0	56.2(1.0)	53.0(1.0)	56.3(0.4)	35.6(1.3)	34.1(1.0)	42.9(1.6)
6.0	77.1(1.0)	74.0(1.1)	76.7(1.2)	48.8(2.8)	47.0(1.7)	62.5(2.6)
8.0	92.7(0.5)	89.6(1.5)	94.5(0.6)	61.3(5.9)	60.0(3.0)	80.6(3.8)
10.0	99.1(0.6)	98.5(1.6)	99.5(0.7)	73.5(7.7)	73.4(4.2)	92.8(4.2)
12.0	100.0	100.0	100.0	85.4(7.7)	86.3(4.9)	97.8(2.5)

Figures in brackets = standard deviation (n = 6)

A17a : SERUM THEOPHYLLINE CONCENTRATIONS (ug/ml) OF INDIVIDUAL VOLUNTEERS AFTER ADMINISTRATION OF FORMULA A IN FASTED STATE (dose = 300 mg)

Volunteer / Time (hour)	KI	JE	SL	NA	VK	JA	Average	SEM
0.5	0.40	-	0.31	0.66	0.32	0.04	0.29	0.10
1.0	1.16	0.09	0.82	1.44	0.58	0.18	0.71	0.22
2.0	2.11	0.66	2.13	2.97	1.20	1.40	1.75	0.34
3.0	3.26	1.26	3.08	4.44	2.51	2.54	2.85	0.43
4.0	4.06	2.37	4.08	5.08	3.22	3.78	3.77	0.37
6.0	4.73	4.04	4.26	5.18	4.10	4.76	4.51	0.18
8.0	4.80	5.14	4.07	4.82	4.32	4.93	4.68	0.16
10.0	4.52	5.44	3.74	4.08	4.46	5.18	4.57	0.26
14.0	3.65	4.11	2.63	2.94	4.30	4.83	3.74	0.34
18.0	2.90	2.86	1.85	2.01	3.83	3.99	2.91	0.36
24.0	1.92	1.61	1.16	1.17	2.96	2.64	1.91	0.31
30.0	1.26	0.97	0.63	0.67	1.98	1.58	1.18	0.22
36.0	0.80	0.42	0.33	0.35	1.30	0.94	0.69	0.16

S.E.M. = standard error of the mean

A17b : SERUM THEOPHYLLINE CONCENTRATIONS (ug/ml) OF INDIVIDUAL VOLUNTEERS AFTER ADMINISTRATION OF UNIPHYLLIN IN FASTED STATE (dose = 300 mg)

Volunteer / Time (hour)	KI	JE	SL	NA	VK	JA	Average	SEM
0.5	0.52	0.36	0.36	0.52	0.40	0.48	0.44	0.03
1.0	1.24	0.65	1.25	0.83	0.91	0.87	0.95	0.09
2.0	2.90	1.80	2.73	2.88	1.80	2.44	2.42	0.21
3.0	2.95	3.29	3.14	3.51	2.79	3.77	3.25	0.15
4.0	3.51	5.10	4.25	4.79	3.98	4.94	4.42	0.25
6.0	3.92	5.51	3.25	4.43	4.18	5.43	4.45	0.36
8.0	4.10	5.71	3.08	4.22	4.63	5.77	4.59	0.42
10.0	4.15	5.11	2.84	4.10	5.00	5.40	4.44	0.39
14.0	3.28	3.47	2.13	3.86	4.27	3.96	3.50	0.31
18.0	2.41	2.06	1.24	2.84	3.20	2.77	2.42	0.28
24.0	1.45	1.13	0.61	1.51	2.10	1.79	1.42	0.21
30.0	0.84	0.64	0.27	0.84	1.30	1.06	0.83	0.15
36.0	0.45	0.38	0.14	0.43	0.86	0.67	0.49	0.1

S.E.M = standard error of the mean

A17c : SERUM THEOPHYLLINE CONCENTRATIONS (ug/ml) OF INDIVIDUAL VOLUNTEERS AFTER ADMINISTRATION OF FORMULA A IN FED STATE (dose = 300 mg)

Volunteer / Time (hour)	LM	KN	EY	AM	WI	WW	Average	SEM
0.5	-	-	-	-	0.10	0.08	0.03	0.02
1.0	0.24	-	0.18	0.17	0.19	0.22	0.17	0.03
2.0	0.44	0.05	0.51	0.65	0.38	0.68	0.45	0.09
3.0	1.82	0.79	1.02	1.56	1.11	1.60	1.32	0.16
4.0	3.48	2.03	1.98	2.46	1.86	2.49	2.38	0.24
6.0	5.74	3.46	3.27	3.39	2.63	3.58	3.68	0.43
8.0	6.10	4.09	4.06	3.49	3.47	4.07	4.21	0.40
10.0	6.15	4.27	4.26	3.42	3.81	4.07	4.33	0.39
14.0	5.76	3.49	4.00	2.98	3.43	3.48	3.86	0.40
18.0	4.70	2.73	3.07	2.26	2.62	2.71	3.02	0.35
24.0	3.21	1.68	2.11	1.65	1.84	1.61	2.02	0.25
30.0	2.04	0.84	1.20	1.01	1.15	0.96	1.20	0.18
36.0	1.18	0.29	0.67	0.61	0.63	0.55	0.66	0.12

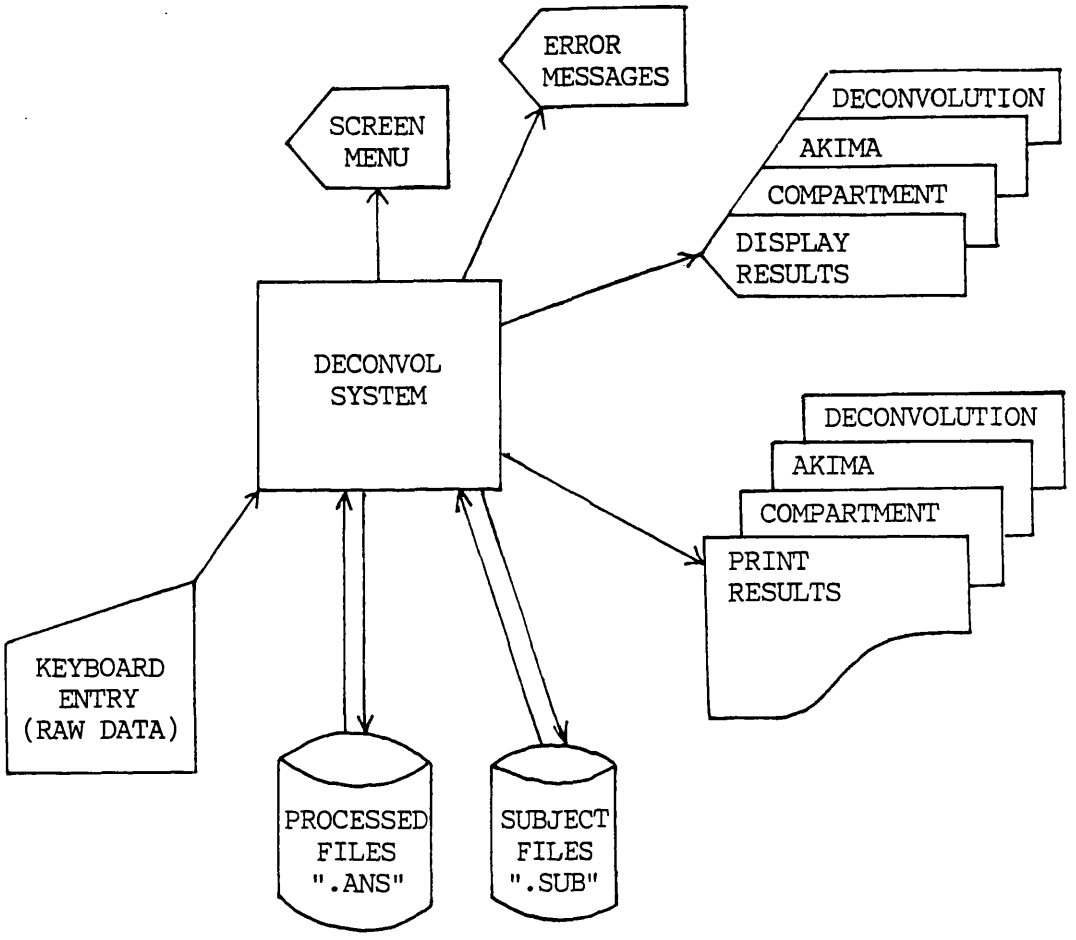
S.E.M = standard error of the mean

A17d : SERUM THEOPHYLLINE CONCENTRATIONS (ug/ml) OF INDIVIDUAL VOLUNTEERS AFTER ADMINISTRATION OF UNIPHYLLIN IN FED STATE (dose = 300 mg)

Volunteer / Time (hour)	LM	KN	EY	AM	WI	WW	Average	SEM
0.5	0.10	0.24	0.36	0.48	0.26	0.43	0.31	0.05
1.0	0.29	0.63	0.66	1.09	0.57	0.65	0.65	0.10
2.0	1.83	1.10	0.99	1.34	1.06	1.33	1.28	0.13
3.0	3.24	2.96	2.52	3.19	2.27	5.03	3.20	0.40
4.0	7.65	9.71	5.33	4.21	4.00	6.70	6.27	0.90
6.0	9.33	8.39	7.38	4.76	4.89	5.82	6.76	0.77
8.0	7.87	6.63	6.25	4.91	4.93	4.96	5.93	0.49
10.0	6.26	5.14	4.99	4.40	4.09	3.97	4.81	0.36
14.0	4.02	3.38	3.26	3.10	2.59	2.38	3.12	0.24
18.0	2.72	2.14	2.01	2.04	1.73	1.39	2.01	0.18
24.0	1.55	1.14	1.16	1.39	0.96	0.83	1.17	0.10
30.0	0.88	0.62	0.62	0.84	0.57	0.47	0.67	0.06
36.0	0.47	0.35	0.36	0.57	0.37	0.28	0.40	0.04

S.E.M. = standard error of the mean

A18a : SYSTEM FLOWCHART FOR DECONVOL SYSTEM

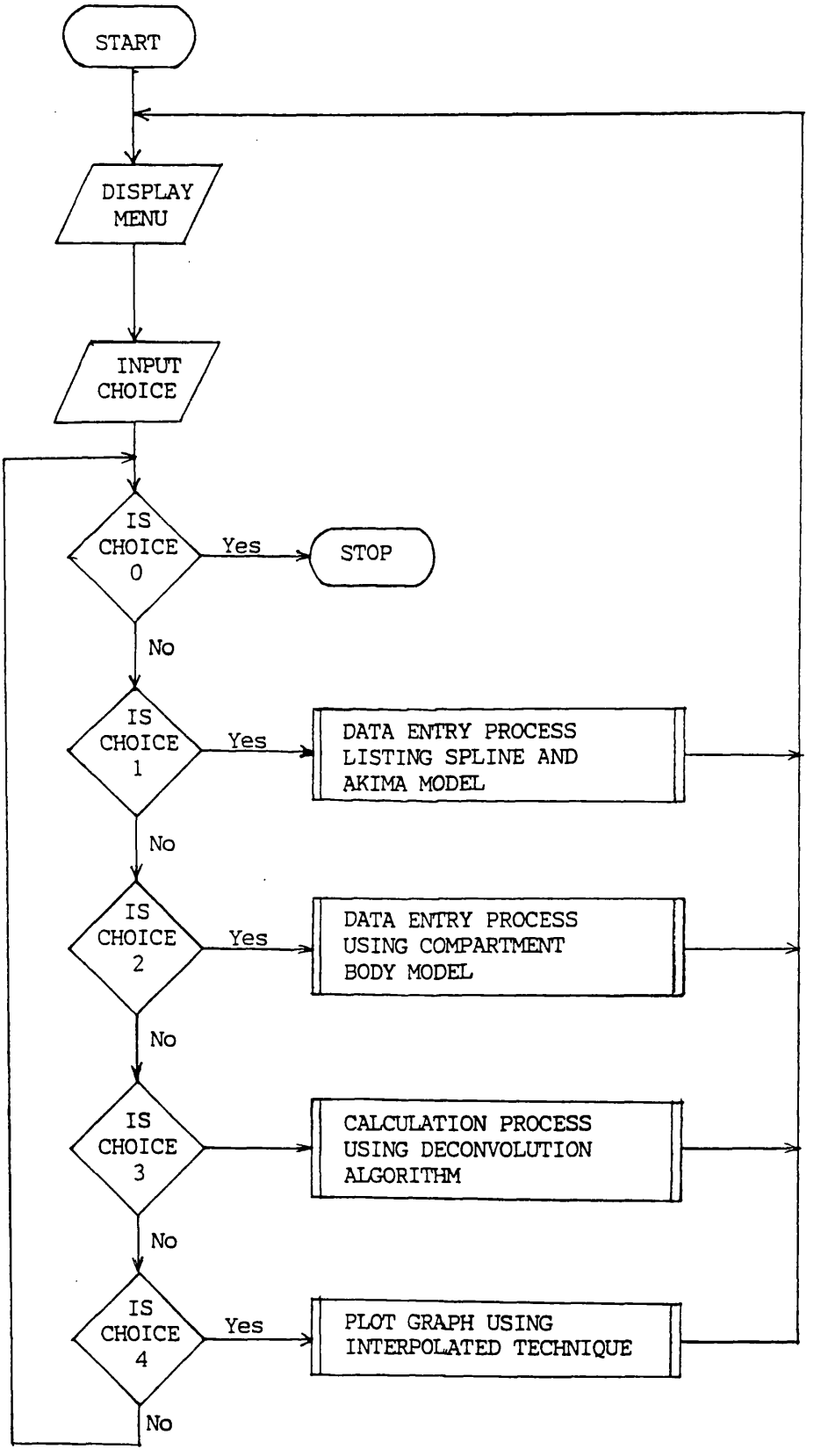


COMPARTMENT = generation of data points from pharmacokinetic parameter values provided. The pharmacokinetic model must be specified

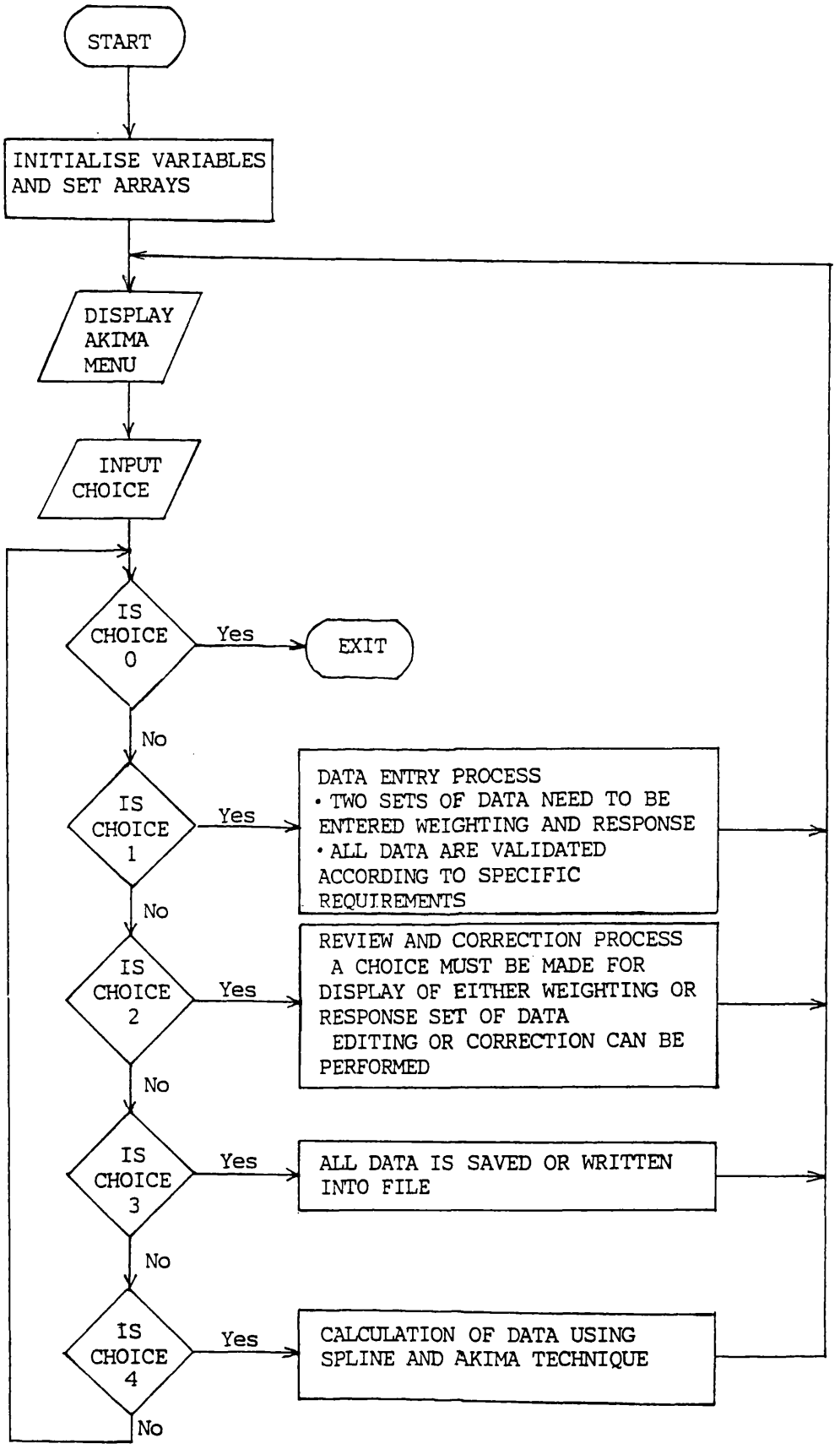
AKIMA = interpolation of data points using spline and akima methods of Fried and Zeitz (1973)

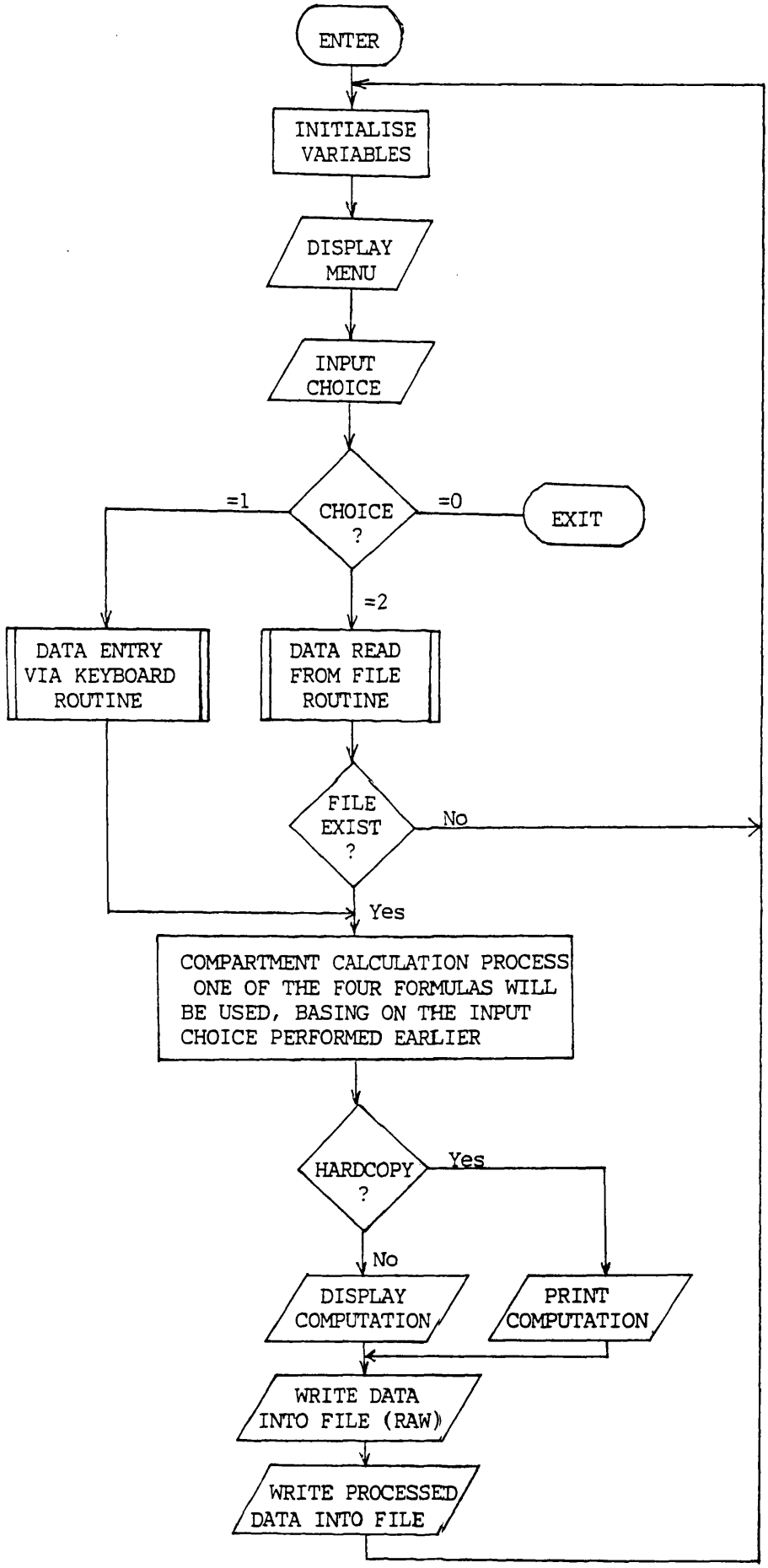
DECONVOLUTION = calculation of in-vivo release/and absorption using the trapezoidal formula of Langenbucher and Moller (1983)

A18b : DECONVOL MAIN PROGRAM FLOWCHART

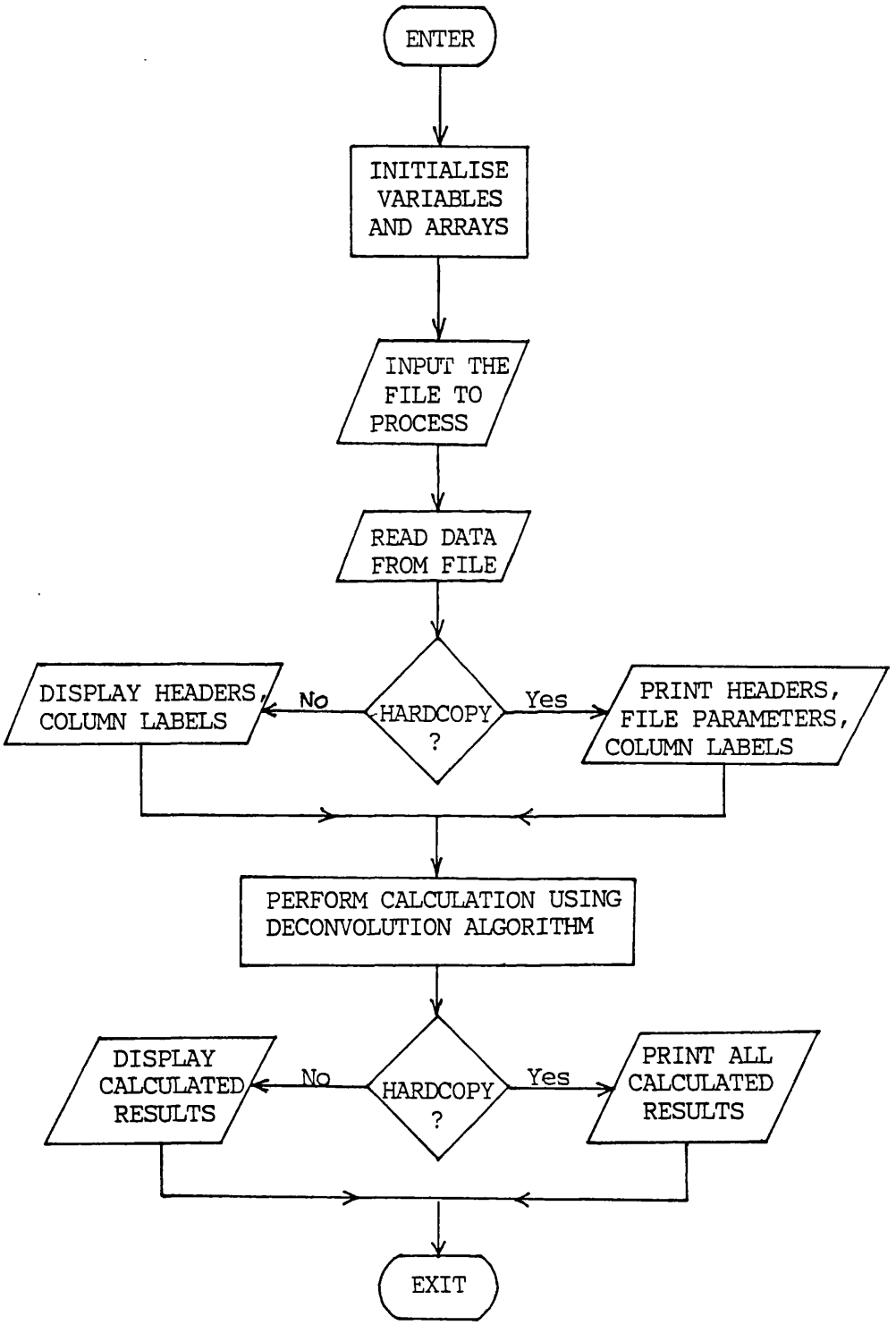


A18c : PROGRAM FLOWCHART FOR AKIMA MODEL

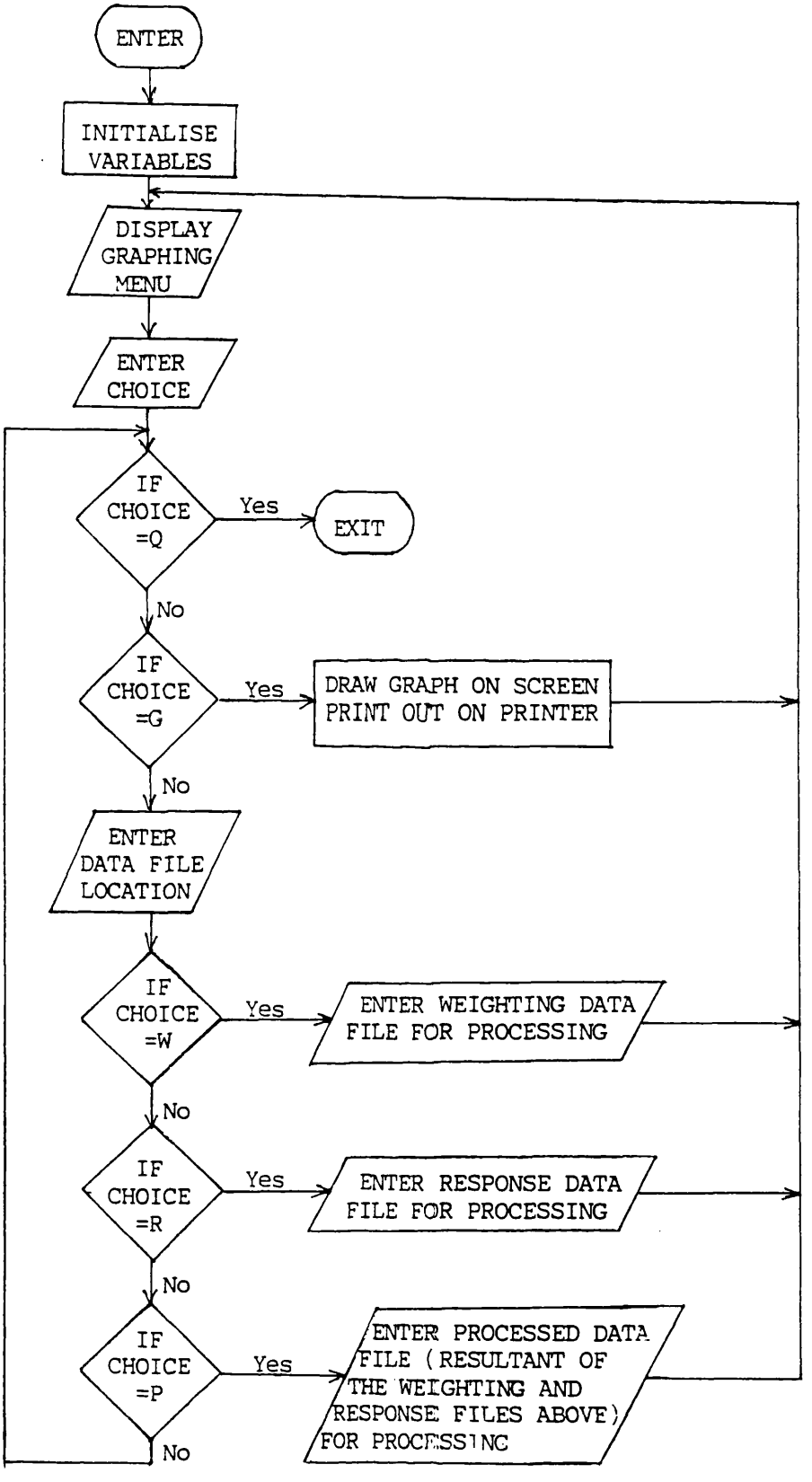



ENTER
INITIALISE VARIABLES
DISPLAY MENU
INPUT CHOICE
CHOICE ?
=1
=0
EXIT
=2
DATA ENTRY VIA KEYBOARD ROUTINE
DATA READ FROM FILE ROUTINE
FILE EXIST ?
No
Yes
COMPARTMENT CALCULATION PROCESS ONE OF THE FOUR FORMULAS WILL BE USED, BASING ON THE INPUT CHOICE PERFORMED EARLIER
HARDCOPY ?
Yes
No
DISPLAY COMPUTATION
PRINT COMPUTATION
WRITE DATA INTO FILE (RAW)
WRITE PROCESSED DATA INTO FILE

A18e : PROGRAM FLOWCHART FOR CALCULATION USING DECONVOLUTION ALGORITHM



A18f : PROGRAM FLOWCHART FOR PLOT GRAPH



A19 : NUMERICAL VALUES OF PHARMACOKINETIC PARAMETERS ESTIMATED FROM THEOPHYLLINE SOLUTION

Volunteer	Ke (hr^{-1})	$t_{\frac{1}{2}}$ (hr)	Ka (hr^{-1})	Vd (L)	AUC_0^{∞} (ug hr ml^{-1})
1	0.11(0.01)	6.48(0.87)	6.91(1.70)	30.1(1.7)	80.5
2	0.08(0.01)	8.97(1.08)	1.23(0.17)	23.8(1.4)	134.1
3	0.08(0.01)	8.61(0.87)	1.56(1.19)	29.5(1.4)	109.5
4	0.08(0.01)	9.07(1.02)	2.76(0.41)	29.5(1.4)	106.8
5	0.06(<0.01)	11.13(0.61)	5.36(0.45)	24.9(0.5)	167.7
6	0.06(<0.01)	10.87(0.62)	6.97(0.68)	35.2(0.7)	118.1
7	0.05(<0.01)	12.73(1.09)	2.99(0.32)	29.7(0.9)	165.1
8	0.07(<0.02)	9.33(2.50)	4.94(2.05)	27.6(2.9)	132.5
9	0.08(0.01)	8.30(1.02)	5.49(1.10)	29.2(1.5)	111.6
10	0.10(0.01)	6.77(0.83)	4.99(0.99)	28.2(1.5)	90.6
11	0.08(<0.01)	8.90(0.34)	3.67(0.20)	29.6(0.5)	107.9
12	0.11(0.02)	6.38(1.02)	2.30(0.49)	26.7(2.1)	92.3
Mean	0.08	8.96	4.10	28.7	118.1
SEM	< 0.01	0.56	0.57	0.83	7.9

Figures in brackets = standard deviation

A20

VOLUNTEER INFORMATION SHEET

In this study we wish to investigate the absorption characteristics of a sustained (long acting) oral drug (theophylline) administered in a gelatin capsule. Theophylline is a drug that has been extensively used for treating reversible airway obstructive diseases such as asthma. Its use in a long acting form has been shown to improve its effectiveness in treating such diseases.

In order to investigate the fate of the drug and its location within the gut, we will give small pellets (1mm) of the drug mixed with similar size lactose pellets labelled with a radioactive isotope (Technetium-99m) in the gelatin capsule. This type of capsule will be administered once. A commercial preparation (Uniphyllin) containing the same drug will be administered on a separate occasion, the dates to be agreed with the volunteer. The commerical preparation will not be labelled with the radioactive isotope.

The amount of radioactive administered on a single occasion is about one-fiftieth (1/50) of the level of dose used in routine clinical imaging investigations.For a single drug administration within 12 months, the absorbed radiation dose resulting from the study is less than 2% of the yearly dose limit for the whole-body of an individual member of the public as stipulated in the ionising Radiations Regulations 1985 (Schedule I).Full details of the radiation dosimetry and the drug theophylline are available on request.

This study has been approved by the Joint UCH/UCL committee on the ethics of clinical investigations (no. 2187).

The volunteers will be non-smoking males between the ages of 18-55 years. They will be required to fast overnight. Lunch and dinner will be provided at 1pm and 7pm.A double-headed gamma camera will be used to image the radioactive pellets with the volunteer sitting between the two camera heads for an initial 90 minutes and then 1/2 hourly intervals until 12 hours after drug administration. We will also be taking ten 5ml blood samples during the course of each drug administration via a small cannula sited in a vein in the forearm.

VOLUNTEER CONSENT FORM

This study is to investigate the absorption of a long acting theophylline formulation in relation to its location in the gut after oral administration.

Theophylline pellets will be taken together with lactose pellets labelled with a radioactive isotope in a gelatin capsule on one occasion, and Uniphyllin on another. Images of the pellets will be taken at intervals over 12 hours using a gamma camera. In addition, 5 millilitres blood samples will be taken at various timed intervals after taking the drug.

For a single drug administration within 12 months, the absorbed radiation dose is less than 2% of the yearly dose limit for the whole-body of an individual member of the public as stipulated in the Ionising Radiations Regulations 1985 (Schedule I).

I have read and understood the nature of this experimental study (Volunteer Information Sheet) and hereby give my consent to participate.

Volunteer	Witness
Name: _____	Name: _____
Signature: _____	Signature: _____
Date: _____	Date: _____

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